Inflammatory mediators in wound healing

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Inflammation is crucial to the highly orchestrated response to tissue injury. Skin wound healing classically has been organized in a progression of overlapping processes: hemostasis, inflammation, fibroplasia/proliferation, and wound remodeling. In this schema, inflammation can be characterized as a circumscribed stage of wound healing that would suggest its effects end as the next stage begins. The latest understanding indicates, however, that inflammation persists throughout all wound healing phases, stimulating and coordinating the essential functions of wound repair. As the science of wound healing progresses, investigators continue to develop new insights and possible therapies for wound healing enhancement.

Although inflammation has been recognized as an early participant in wound healing, the realization of the importance of inflammation and its regulatory role in wound healing has been a gradual process. The connection between inflammation and wound healing can be traced back to ancient Egypt. Ancient papyruses from circa 1400 BC document Egyptian physicians placing a therapeutic paste of honey, grease, and lint into open wounds to remove skin and wound pus and to encourage healing [1]. Honey, which was used extensively in Egyptian medicine, presumably derived healing properties from its hypertonic nature and ability to draw fluid from a wound and its activity as an antiseptic [2,3]. These ancient techniques are viewed as the first documented efforts to control the by-products of inflammation. The understanding of the role of inflammation and infection in wound healing in ancient times was limited, however. Healers of that time also promoted that it was good for a wound to “rot a bit” [4]. This erroneous conclusion...
continued throughout the Middle Ages and Renaissance and is noted in physicians’ description of “laudable pus” [2], suggesting that pus was visible documentation of the body’s increased efforts to heal a wound.

This teaching first was refuted by physicians such as Villanova of Spain, who taught “a collection of pus is best dissolved by incision and cleaning out the purulent material” [5]. The detrimental effects of uncontrolled infection and inflammation were not understood until the modern age, in which Pasteur and Lister described the germ theory of infection and its prevention by the antiseptic technique. Metchnikoff, the Russian pathologist who shared the 1908 Nobel Prize in medicine with Ehrlich, further defined inflammation with his discovery of phagocytosis and the theory that the purpose of inflammation was to bring phagocytic cells to the injured area to engulf invading bacteria [6].

During the twentieth century, numerous investigators characterized the role of leukocytes and their products in the response to injury and infection. Initially, these events were not thought to have a direct effect on the later proliferative and remodeling phases of wound healing. Since the 1970s, however, wound healing research has shown a regulatory relationship between inflammation and wound healing. Correspondingly, many new inflammatory mediators (IMs) have been identified. This article reviews current understanding of the relationship between inflammation and wound healing as it primarily relates to its control by IMs.

**Terminology**

The mediators that initiate inflammation in healing wounds are soluble factors released by resident cells of the wound bed and by platelets and leukocytes delivered by the circulation after the disruption of intact skin. These soluble factors initiate a series of events that attempt to stabilize the wound, remove invading organisms, and return the wound to preinjury architecture. The ability of wound healing mechanisms to achieve these goals largely depends on the production, regulation, and control of IMs.

The distinction of what exactly constitutes an IM is confusing. Inflammation is propagated and controlled by many different soluble factors, including but not limited to cytokines, growth factors, proteases, eicosanoids, kinins, and cellular metabolites. Confusion results from attempting to classify rigidly these substances that have multiple and overlapping effects and that are released from different cellular sources. By applying some simple and consistent rules, however, IMs can be organized in a manner to define more clearly their roles in wound healing. This discussion also explores the distinction between two groups of the most extensively investigated wound healing soluble factors: cytokines and growth factors.

Cytokines are small regulatory peptides or glycoproteins of molecular weight of 5 to 30 kd that are released by practically all nucleated cells [7].
Cytokines are extremely potent and are active at picomolar-to-nanomolar concentrations. They usually act within a short distance of their release as intracrine, autocrine, or paracrine signals. They are released transiently to modulate immune or repair processes by controlling cellular growth, differentiation, metabolism, and protein synthesis [2,8–14]. A key aspect of cytokine biology is that an individual cytokine can elicit varying responses in many different types of cells. This *pleiotropic* property is amplified by cytokine redundancy. That is, cytokines, even those from different family classifications, have overlapping abilities to stimulate the same cellular functions [15]. Fibroblast proliferation is stimulated by transforming growth factor (TGF)-β and interleukin (IL)-1, angiogenesis is activated by epidermal growth factor (EGF) and IL-8, and neutrophil wound infiltration is triggered by tumor necrosis factor (TNF)-α and neutrophil-activating peptide (NAP)-2. This pleiotropy underscores one of the fundamental tenets of wound healing: although it is a highly orchestrated process, redundancy creates a backup for this important homeostatic process.

The terms *cytokine* and *growth factor* often have been used interchangeably with resulting confusion. These are two separate categories of signaling proteins that are distinguished by their methods of action and cellular targets. The distinction is tenuous but best described by Vilcek [15], who characterized growth factors as constitutively present mediators that are less tightly regulated and have as targets nonhematopoietic cells. Cytokines are related more directly to control of immune cell responses. The growth factors that play essential roles in wound healing, such as platelet-derived growth factor (PDGF), EGF, fibroblast growth factor (FGF), and vascular endothelial growth factor (VEGF), can be referred to as *connective tissue growth factors* given their function locally and rare systemic effects [15]. A notable exception in nomenclature is TGF-β. TGF-β, which is called a *growth factor*, is classified better as a cytokine rather than a connective tissue growth factor because of its smaller molecular weight and its selective effect on multiple inflammatory processes. The prohealing properties of TGF-β, as seen in its ability to reduce incisional hernias in rats, can be attributed to its induction of chemotaxis by inflammatory cells as much as its enhancement of fibroblast collagen production [16].

Another group of signaling proteins that has been confused with the term *growth factors* is factors such as insulin and somatomedin. These proteins are classified best as endocrine hormones and are distinguished from connective tissue growth factors by their origins from cells of specialized glands dedicated to their production and their systemic effects by bloodstream travel. These types of growth factors have little direct effect on wound healing and are not discussed in this article.

Cytokines can be subcategorized further as chemokines, lymphokines, monokines, ILs, and interferons (IFNs) [7,17,18]. Chemokines are a subset of cytokines and refer to proinflammatory soluble factors that have the ability to attract and activate leukocytes. Lymphokines, monokines, and ILs
are classifications to distinguish chemokines further. Lymphokines and monokines are defined as soluble factors produced by activated T lymphocytes and mononuclear phagocytes [8]. The term *interleukins* originally was designed to refer to factors secreted by one type of leukocyte that act on other leukocytes; now it is known that these mediators have a plethora of effects on and are released from nonhemopoietic cells as well. The current nomenclature that assigned the ILs numbers was devised in Interlaken, Switzerland, in 1978 to replace the original eponyms that either described the cell origin of first discovery or the action first detected [7]. Given the fact that these cytokines subsequently were determined to have multiple functions, a progressive numbering system was instituted, currently IL-1 to IL-18.

Chemokines are subdivided further into four families characterized by a conserved amino acid pattern at the first two cysteine residues near the N-terminus [19]. The significance of this amino acid pattern is that chemokines with different cysteine patterns chemoattract different types of leukocytes. The first group (α-chemokines) contains chemokines that have a pattern of two cysteine (C-C) residues separated by a nonconserved residue (-X-) and designated *CXC chemokines*. The CXC chemokines are distinguished further by the sequence glutamic acid-leucine-arginine near the N-terminal preceding the CXC sequence (Fig. 1). CXC chemokines with this ELR motif attract neutrophils only; CXC chemokines without the motif attract activated lymphocytes [19]. The ELR CXC chemokines important in wound healing include IL-8, growth-related oncogene (GRO)-α, GRO-β, GRO-γ, NAP-2, and epithelial neutrophil-activating protein (ENA-78). The wound healing–significant non-ELR CXC chemokines are IFN-inducible protein (IP-10) and monokine induced by γ-IFN (MIG).

The second family, *C-C chemokines* (β-chemokines), has two adjacent cysteines that are chemoattractant for lymphocytes, monocytes, eosinophils, and basophils but not neutrophils and includes monocyte chemoattractant proteins (MCP)-1–5, regulated on activation normal T cell expressed and secreted (RANTES), and macrophage-derived chemokine (MDC). The last two families have a lone cysteine residue and a pair separated by three amino acids, C and *CXXXC chemokines*. These groups have only one classified chemokine apiece and have yet to be found to play a major role in wound healing [19,20].

Hematopoietic colony-stimulating factors (CSFs) are a subset of cytokines that also have stimulatory wound healing effects. Macrophages secrete CSF-1, which aids self-preservation and acts as an autocrine mediator. On activation, macrophages release granulocyte-macrophage (GM)-CSF, which has chemotactic and generalized cellular proliferative and activation properties [21]. Fig. 2 summarizes the organization of the IM classifications.

The IMs currently known to be crucial to the wound healing process are IL-1, IL-2, IL-4, IL-6, IL-8, GM-CSF, G-CSF, macrophage (M)-CSF,
Chemokine protein structure provides the key to its function. (A) C chemokines contain only one cysteine residue (C) and stimulate T lymphocytes. (B) CC chemokines contain two adjacent cysteine residues and are important in monocyte chemotaxis. (C) CXC chemokines are separated into two groups, one containing the ELR moiety, which attracts neutrophils, and one containing the non ELR, which acts on lymphocytes. (D) CXXC chemokines are associated with natural killer cell activation.
Fig. 2. Soluble factors of wound healing. Inflammatory mediators consist of a subset of the soluble factors that control wound healing. Platelet-derived growth factor (PDGF) can be considered an inflammatory mediator given its significant inflammatory properties. *Except IL-8, which is classified as a CXC chemokine. See text for other abbreviations.
macrophage inflammatory protein-1 (MIP), MCP-1, NAP-2, IP-10, IFNs, TGF-β; TNF-α, platelet factor 4 (PF4), and PDGF. PDGF classically is classified as a connective tissue growth factor but contributes significantly to inflammatory stimulation and shares a place in both categories. These IMs have effects throughout all stages of wound healing and are key factors that orchestrate the release of other cytokines, growth factors, extracellular matrix (ECM) proteins, and proteases (Table 1).

**Inflammatory mediators in normal wound healing**

**Inflammatory mediator cascade**

The earliest acute wound inflammatory signals are released after platelet degranulation and from the traumatized cells at the disrupted edges of the skin wound. Hemostasis and inflammation are stimulated simultaneously by the release of multiple soluble factors. Platelet α-granule soluble factors include the following:

- PDGF
- TGF-β
- FGF
- EGF
- β-Thromboglobulin

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PDGF, PF4, and TGF-β are the most relevant wound healing factors released from platelet α granules immediately on wounding. These three factors remain elevated throughout all the stages of normal wound healing. After thrombus formation, chemokine–connective tissue activating peptide III (CTAP-III), which also is released from platelets, is converted to NAP-2 by neutrophils attached to the thrombus. NAP-2 mediates one of the first signals for neutrophil chemotaxis [22].

From the resident keratinocytes at the wound edge, MCP-1 is released on skin disruption and is expressed almost exclusively in the first week of wounding [23]. MCP-1 levels rise within the first 12 hours, peaking at 1 day, and a return to baseline levels by day 7 [23,24]. Shortly after release of MCP-1, IL-1 and TNF-α are released from disrupted endothelial cells. Their levels peak within the first day of wounding and stimulate multiple inflammatory processes and modulate wound regeneration [25]. Early in the wound process, IL-1 and TNF-α chemoattract multiple inflammatory cells and stimulate them to secrete further cytokines and growth factors. Specifically, TNF-α and IL-1 trigger the release of IL-8, whose expression peaks early in the wound process at around 7 hours [22]. IL-8, which has been shown to enhance reepithelialization in vivo [26], also chemoattracts neutrophils. IL-8 expression is timed to increase at the point at which NAP-2-stimulated neutrophil chemotaxis reaches its limit, continuing neutrophil wound infiltration. IL-6, important in inhibiting ECM breakdown during proliferation [27], is expressed temporally parallel to IL-8. Both mediators reach peak concentration in the wound at 24 hours, then decline locally, but these IMs remain elevated systemically into the final stages of wound healing [26].

IL-4 stimulation of fibroblast proliferation begins within the first 10 days of wounding [28]. IL-10 exhibits bimodal wound levels with concentrations peaking at 3 hours after wound incision, returning to normal, with another peak at 72 hours. IP-10 shows peak expression after day 4. Fig. 3 graphically displays the temporal relationship between these multiple factors.

Hemostasis

To achieve hemostasis, the coagulation cascade is initiated, and platelet α granules degranulate, releasing large quantities of TGF-β in the early wound process. TGF-β stimulates the chemotaxis of macrophages and lymphocytes.
Fig. 3. Temporal expression profile of selected inflammatory cytokines throughout the wound healing timeline. See text for abbreviations.
and augments their proliferation [13,29,30]. Lymphocytes and monocytes are chemoattracted to the wound by other platelet-derived inflammatory products, such as PDGF, TGF-β, PF4, complement protein C5a, leukotriene B4, and platelet-activating factor (PAF) [10,13,31,32].

The fibrin provisional matrix of the resolving thrombus itself plays a role in the inflammatory response. It serves as a protein reservoir by binding cytokines and growth factors, amplifying their chemotactic properties by increasing local concentrations and directing cellular infiltration to the wound site [33]. MCP-1, as noted, is tightly associated with the newly formed thrombus. MCP-1 levels are shown to increase during natural resolution of venous thrombus, and increased organization and resolution of thrombi can be elicited by MCP-1 local augmentation [34].

At this time in the healing cascade, the IMs have begun stimulation of the various cell types necessary for wound healing to proceed. The wound also has been primed for inflammatory cell infiltration.

**Cellular infiltration**

The most defined function of wound IMs is chemoattraction of inflammatory cells. In the early stages of wound healing, there is constant and increasing trafficking of leukocytes to the wound bed. Neutrophils are the first inflammatory cells to reach the wound scene and are attracted by many different soluble factors. As discussed earlier, the ELR CXC chemokines (IL-8, GRO-α, GRO-β, GRO-γ, NAP-2, and ENA-78) have specific neutrophil chemoattractant ability [7,19,20] and are released during initial wounding. TNF-α and IL-1 (ubiquitous cytokines of the early healing wound with similar function and stimulatory targets) are released shortly afterward from endothelial cells and produce an ample and sustained neutrophil and lymphocytic chemotaxis. Neutrophil activity and infiltration shows a highly preserved quality in that its chemotaxis extends also to connective tissue growth factors. FGF and PDGF stimulate neutrophil wound infiltration [14,35,36].

In the early stages of inflammation, the recruited neutrophils quickly become the dominant leukocyte in the wound. Although the primary function of neutrophils in this condition is microbial clearance, neutrophils exhibit wound healing–enhancing abilities by producing angiogenic factors VEGF, TNF-α, and IL-1 [37]. Neutrophils also express proteinases that are involved in degrading the matrix of the wound bed, enabling further cellular infiltration by macrophages, fibroblasts, and keratinocytes [38]. Although neutrophils play an essential role in wound healing, they may not be crucial to the progression of wound closure, given that wound healing can still occur in their absence [14].

Incursion of monocytes into the wound and their transformation into macrophages begins at day 1 and increases rapidly so that these cells replace neutrophils as the predominate leukocyte by day 3 [35]. Monocytes are
chemoattracted to the wound by a wide variety of factors. IMs from the CC chemokine family—in particular, MCP-1 and MIP-1—and TGF-β, IL-1, and TNF-α are powerful stimulants of monocyte infiltration [36,39–42]. Adjunct inflammatory factors, such as leukotrienes, complement C5a, and bacterial products such as lipopolysaccharides, also play a supportive role in monocyte and neutrophil attraction [22]. Noninflammatory mediators also contribute significantly to monocyte and neutrophil chemotaxis. Connective tissue growth factors FGF and PDGF [14,35] and coagulation by-products, such as fibrinopeptides, collagen fragments, elastin, and activated thrombin, further stimulate neutrophil and monocyte immigration to the wound [42,43].

Other inflammatory cells play a role in wound healing. Lymphocytes are recruited to the wound by non-ELR CXC chemokines and release inflammation-boosting cytokines, such as IFN-γ and IL-1. Lymphocytes are capable of producing EGF and FGF [44] and secrete matrix metalloproteinases (MMPs) after stimulation by MIP-1 and RANTES [45]. Even mast cells, which are known for their key role in hypersensitivity reactions, are recruited to the wound site and activated to release IL-4, a cytokine that stimulates fibroblast proliferation [28].

Monocytes mature into macrophages through activation by IL-2, TNF-α, IFN-γ (released by T lymphocytes), and PDGF [32,42]. These activated macrophages become the orchestrator of the wound and secrete more than 100 different substances, many of which are cytokines and growth factors that further stimulate the inflammatory process and upregulate wound repair systems [38,46–48]. TNF-α and IL-1, in particular, are released to increase inflammatory adhesion molecule production (eg, ICAM, ECAM), modifying thrombogenicity, recruitment and activation of neutrophils, and autocrine activation of macrophages [2,8,49]. TNF-α and IL-1 stimulate macrophages to express other cytokines, including IL-6, IL-8, GM-CSF, G-CSF, MCP-1, and autosecretion of IL-1. In addition to cytokine secretion, macrophages secrete proteinases that digest damaged wound bed matrix and allow migration by other connective tissue cells [50,51].

Matrix degradation by MMPs facilitates migration of fibroblasts, endothelial cells, and other mesenchymal cells that propagate during the proliferative stage of wound healing. This third wave of cellular infiltration is regulated by a diverse group of factors. Some cytokines, such as PDGF, TGF-β, and IL-1, which are chemotactic for leukocytes in early inflammation, also chemoattract mesenchymal cells. EGF [52], lymphokines [53], collagen peptides [54], and fibronectin [55] also have been shown to be chemotactic for fibroblasts. Because fibroblasts also secrete collagen and fibronectin, mesenchymal cells perpetuate their own chemotaxis.

Not all inflammatory cell mediators act as pure stimulants of inflammation. IL-10 and IL-4 downregulate expression of other inflammatory cytokines [22,56]. This downregulation can be correlated with the late increase of IL-4 in the wound. As noted before, IL-4 has a bimodal
concentration profile in the wound. The later peak at 72 hours can be correlated with a need for downregulation of inflammatory processes and IL-4 suppression ability. IL-4 also inhibits further chemokine release in vitro at higher levels, suggesting a dose-dependent inflammatory limiting mechanism [57].

Early wound remodeling

Significant structural changes occur throughout the wound healing process. Preparation for the cellular influx occurs in the early phases as the provisional matrix is restructured. Many enzymes are increased within the early wound because of the induction by cytokines and growth factors. MMPs comprise the core of wound enzymes, although plasminogen activator also is involved in wound healing [58]. Neutrophils and macrophages secrete many MMPs and collectively play a role in collagen degradation. Neutrophils and macrophages have been shown to secrete MMP-8 (neutrophil collagenase) and MMP-9 (92 kd gelatinase) [59], and interstitial macrophages have been shown to produce MMP-1 (collagenase-1) and MMP-3 (stromelysin) [60]. In addition to ECM degradation, macrophages use proteinases to phagocytize and digest stromal collagen fibers [61]. The activity of these enzymes not only begins the process of prewound skin architecture formation, but also opens pathways for further inflammatory and later mesenchymal cell infiltration.

Investigations now propose that MMPs also can affect inflammatory reactions by cleavage of chemokines. MMP-2 (gelatinase A) digests MCP-3 and dampens the inflammatory response by inhibiting its chemotactic properties [62]. Because MMP-2 and MMP-9 are elevated significantly in chronic wounds [63], MMP degradation of mediators is a possible cause of delayed repair.

Cytokines produced by macrophages also regulate MMP production in fibroblasts, the primary cell involved with matrix deposition and reorganization. The effect that cytokines have on MMP expression and activation depends on the proteinase and the specific IM [64]. TGF-β induces expression of pro-MMP-9 in keratinocytes, and activation is triggered by TNF-α [65]. In contrast to MMP-9, MMP-2 is constitutively present, and its activation requires stimulation by TNF-α and collagen [66]. IL-1 and PDGF also stimulate fibroblast MMP production [67,68]. Suppression of fibroblast MMP-3 has been shown by IFN-γ. An additional level of control of MMP activity is provided by tissue inhibitors of metalloproteinases (TIMPs) that are secreted by TGF-β-stimulated and IL-6-stimulated fibroblasts [68]. Tissue-type plasminogen activator and urokinase-type plasminogen activator are upregulated in keratinocytes during reepithelialization [58]. IL-8 induces urokinase-type plasminogen activator in keratinocytes, a fact that may be relevant to this cytokine’s ability to stimulate keratinocyte migration [69]. Not all IMs are stimulatory.
IL-6 has been shown to induce TIMP production [27]. These factors inhibit MMP function and counterbalance ECM digestion.

Reepithelialization

Concurrent with inflammatory cell and fibroblast wound infiltration, reepithelialization occurs to recreate the natural skin barrier. Within hours of wounding, basal keratinocytes from the wound edges begin to migrate laterally to cover the defect [13,43]. Keratinocytes on the wound border are exposed to serum for the first time that contains a plethora of new inflammatory cytokines and connective tissue growth factors. This new exposure to increased amounts and varieties of IMs stimulates new properties in the keratinocyte that are theorized to promote reepithelialization [70]. Keratinocyte motility in the wound is a complex process, and the signal that initiates it is not defined clearly. Inflammatory soluble factors play a pivotal role, however.

Although cytokines and growth factors seem to aid keratinocyte motility, their actions are not the only migration-initiating factor. Keratinocyte motility is sensitive to the ECM environment. Collagen types I and IV, fibronectin [71], and vitronectin [72,73] all seem to facilitate Keratinocyte migration. Collagen in the absence of cytokines can drive keratinocyte migration (Henry G, et al, submitted for publication, 2003). In contrast, laminin-1 and laminin-5 prevent keratinocyte migration regardless of growth factor and cytokine exposure [74, 75]. Cytokines play an essential assistive role in cell motility. TGF-α, TGF-β, IL-1, and IL-8 all stimulate keratinocyte migration [17,30,76–80]. Most but not all of these agents also stimulate keratinocyte proliferation. These processes still must have some degree of independence because TGF-β, which stimulates motility, also is shown to inhibit proliferation [79,81]. Although many IMs have been shown to promote reepithelialization, experimentally single soluble factors do not induce keratinocyte migration to the degree of serum [70]. Synergistic cytokine combinations are likely to be important as inducers of wound reepithelialization.

Fibroplasia and proliferation

The infiltration of fibroblasts into the wound begins the first day after wounding, and fibroblasts become the predominate cell type by day 4 [10,13,43]. Fibroblast recruitment is induced by inflammatory cytokines, such as IL-1, and connective tissue growth factors including FGF and PDGF [14,35,36]. Wound collagen content starts to increase concurrent with fibroblast infiltration and is stimulated by multiple cytokines, the most significant being TGF-β. TGF-β stimulates fibroblast collagen production and prevents collagen degradation by triggering TIMP secretion [82,83]. TGF-β also increases fibronectin synthesis [83]. The interaction between
secreted ECM proteins and migrating cells is augmented by TGF-β modulation of integrin receptor expression to increase cellular adhesion to matrix proteins [84]. The PDGF mechanism in fibroplasia exemplifies the overlapping nature of wound healing as it indirectly increases proliferation by stimulating fibroblast autocrine production of TGF-β [85].

New studies have shown that CC chemokines too can stimulate inflammatory cells indirectly to produce ECM. MCP-1 induces TGF-β release from fibroblasts, which have an autocrine function to increase fibroblast matrix protein production [34]. Inflammatory cells also play a role in matrix deposition; monocytes have been shown to deposit collagen in the wound bed when activated by ILs [86].

As with all aspects of wound healing, the signal to turn off an activity is as important as the signals to stimulate. IP-10 inhibits EGF-induced fibroblast motility [87]. Because it is expressed in the late phases of wound healing (after day 4), it may have a role to limit recruitment of fibroblasts and prevent proliferative scar [23]. PF4 is present throughout wound healing and has negative mitogenic effects on fibroblasts [88]. The lack of this factor could play a role in the excessive function in hypertrophic scarring or keloid formation.

**Wound contraction**

Wound contraction is an essential aspect of wound healing that decreases the area of the wound defect, promoting easier wound closure. Wound contraction is performed by fibroblasts as they attach to and migrate through provisional matrix. TGF-β and PDGF stimulate these myofibroblasts through independent mechanisms to contract collagen lattices in vitro [89,90]. IL-8 has been shown to inhibit fibroblast contraction of collagen lattices and to cause disorganization of fibroblast microfilaments and overall morphology [91]. This inhibition persists for several days in vitro, suggesting that cells retain a “memory” to chemokine exposure [92]. If this response is true in situ, it gives a specific mechanism for the fact that inflamed wounds do not heal well by contraction.

**Late wound remodeling**

Wound remodeling and fibroplasia are as highly orchestrated operations as the earlier phases of wound healing. The restructuring of the newly synthesized tissue is due to differential regulation of the production of new ECM, its degradation by proteases (primarily by MMPs), and the inhibition of proteinases by TIMPs. Although diverse cell types produce these proteins, most and the workhorse of remodeling are the fibroblasts. Many of the inflammatory cytokines that were released in the beginning of wound healing still are present in the remodeling phase and play key roles in its progression. Remodeling becomes the predominant process at the point at which the amount of collagen in the wound becomes constant, occurring at
around 21 days after wounding. At this time, collagen degradation matches production as wound restructuring attempts to approximate the pre-wounded architecture.

Tissue reorganization is regulated by the balance between matrix synthesis and the proteinases that remove them. The effects of inflammatory cytokines on these changes in connective tissue are complex. Phan and colleagues [93] reported increased TGF-β expression by endothelial cells after stimulation by TNF-α and IL-1. In another report, TNF-α downregulates fibroblast collagen synthesis by suppressing TGF-β expression [94]. During this period, other ECM essential to the earlier formed provisional matrix, such as fibronectin, is degraded as collagen synthesis continues [95].

Matrix remodeling proteinases, MMP-1, MMP-2, MMP-9, and MMP-3, are detected in the acute and healing wound and are under the influence of many cytokines, including TGF-β [96], PDGF [97], IL-1 [98], and EGF [99]. TNF-α and IL-1 have significant effects on the activity of MMP-2 and MMP-9. The authors reported on the differential regulation of MMP-2 by TNF-α [66] and compared it with MMP-9 regulation by TNF-α and TGF-β [69]. These differences likely allow changes in MMP activity in the wound as matrix and cytokine concentrations change during normal healing. An additional level of influence by IMs occurs through TIMPs. MMP activity is suppressed by TIMPs, whose production in fibroblasts is upregulated by IL-6 [27]. TNF-α stimulates the release of IL-6 by fibroblasts and shows the pleiotropic effects of IMs by decreasing collagen production and inhibiting its breakdown.

**Inflammatory mediators in poor wound healing**

**Chronic wounds**

Although inflammation is crucial to normal wound healing, disordered and unchecked inflammation can lead to deficient healing. Persistent inflammation has long been associated with chronic nonhealing of skin wounds [100–103]. Certain IMs, which play essential prohealing roles in early wound healing, also are associated with defective wound repair if high levels persist throughout the later stages. Elevated levels of TNF-α have been linked to deficient healing [104–106]. In animal models, TNF-α inhibited wound healing by causing insufficient collagen deposition during granulation [107]. Studies performed on surgically delayed mice wounds also showed increased levels of TNF-α, IL-1, and IL-6 [102].

Experimentally, the authors examined inflammatory cytokines from normally healing and nonhealing human burn wounds. Many patients with significant burn injuries develop small areas of unhealed burn wound in situations that normally would be expected to heal quickly by contraction and reepithelialization. The authors quantified the concentration of three inflammatory cytokines in these nonhealing wounds (Table 2). These results document significantly increased levels of IL-8 and TNF-α in
delayed-healing wounds in contrast to wounds that had healed. TNF levels were approximately 12-fold higher in delayed-healing wounds compared with normally healing acute wounds. IL-8 levels also were elevated more than threefold in delayed wounds compared with normal or healed wounds. These findings correlate with other human studies that recorded TNF-α, IL-1, and IL-6 at significantly higher concentrations in wound fluid from nonhealing compared with healing leg ulcers [103]. The causative relationship between these increased cytokine levels and poor healing is complex. IL-8 decreases keratinocyte replication [91] and stimulates migration [108]. IL-8 decreases the ability of fibroblasts to contract collagen [91], whereas chronic wound fluid enhances fibroblast proliferation [109].

TNF-α [65,66] increases the activity of MMP-2 and MMP-9, proteinases that have been associated strongly with wound chronicity [63,110]. IL-1 has been associated with the production of collagenase-1 (MMP-1) [68,111]. Although IL-1 and MMP-1 are part of normal wound healing, they are present at elevated levels in chronic compared with acute wounds [103]. These data suggest but do not prove that the dysregulation of inflammation may induce abnormalities in the subsequent processes of wound healing. Alternatively, IMs themselves may not cause poor wound healing, but their elevated levels may be a sign of homeostatic response attempting to correct the underlying wound pathology.

In contrast to the IMs discussed earlier, TGF-β is not elevated in poorly healing wounds. Chronic wound fluid has been shown to contained decreased TGF-β concentration compared with acute wounds [112]. Because TGF-β is associated with hyperfibrotic disorders, it most likely has a limited role in a chronic wound that has failed to reach the proliferative phase.

**Keloid and hypertrophic scars**

Hypertrophic scar and keloid formations have long been associated with deranged fibroblast function and elevated activity of TGF-β [113–118]. These changes are the result of increased local TGF-β production and result
in increased contraction. Specifically, TGF isoforms β1 and β2 [119] have been associated with excessive scar formation [33]. Fibroblasts obtained from hypertrophic and keloid scars show a higher level of TGF-β1 expression and collagen production [118,119]. In patients with burn hypertrophic scars, the IM production seems to be altered systemically and in the local wound. Analysis of serum derived from patients with proliferative burn scars showed increased levels of IL-1, IL-6, TNF-α, and TGF-β2 compared with serum of unwounded subjects [120]. Consequently, one of the most significant factors involved in hyperproliferative scars is TGF-β.

Therapies aimed at altering TGF-β levels have shown some success in in vitro models. Drug inhibition by tamoxifen has been shown to decrease the TGF-β1 production by keloid fibroblasts [119]. Inhibiting TGF-β2 with blocking antibodies also has been shown to decrease fibroblast collagen lattice contraction [121]. Because TNF-α-treated keloid fibroblasts significantly decrease collagen type I production [122], overproduction of collagen by hypertrophic fibroblasts may be a dysregulation of TNF-α as well. Peruccio and associates [123] showed that altered TNF-α mRNA biosynthesis is present in postburn hypertrophic scars.

Tredget and coworkers [116] showed that IFNs may play an important role in hypertrophic scar regulation. IFNs are antifibrogenic cytokines that decrease the fibrotic phenotype of hypertrophic scar fibroblasts. IFN alfa-2b has been shown to inhibit posttranslational changes to collagen and to increase collagenase activity [124,125]. IFN alfa-2b, IFN-β, and IFN-γ all reduce collagen lattice contraction [126]. Clinically, IFN-γ has shown consistent results in reducing keloid size by intradermal injections [127,128], whereas IFN alfa-2b has produced conflicting results [129,130]. Optimal therapy with these agents requires further research.

**Treatment of wounds with inflammatory mediators**

The role of IMs as adjunct therapy for poor wound healing is challenged by the fact that several IMs already are elevated in wounds that heal poorly. Different wounds heal poorly for different reasons. In some cases, poor healing may be due to inadequate or excessive inflammation. Several studies have tested IM therapeutic potential; the results have been variable yet hopeful. The number of IMs studied as treatment options is small compared with the total number of IMs involved in wound healing, leaving more investigations to be pursued.

Many studies have shown that GM-CSF enhances aspects of wound healing, such as migration and proliferation of endothelial cells, keratinocyte proliferation, macrophage and neutrophil activation, and granulation tissue formation [131–134]. These prohealing properties have resulted in some initial clinical success in treating chronic, refractory wounds and accelerating incisional wound healing [135,136]. Pretreatment of murine skin with GM-CSF before wounding shows priming of the inflammatory reaction. This
application coupled with TGF-β doubles breaking strength of an acute wound [137]. Sequential application of GM-CSF and basic FGF with 1-year follow-up exhibited marked enhancement of pressure ulcer healing compared with control [138,139]. In human trials, preliminary clinical observations showed that intradermal application of GM-CSF alone significantly enhanced healing of recalcitrant diabetic foot ulcers (personal experience).

Other hematopoietic growth factors have been used to improve wound healing. M-CSF accelerated wound healing in nonischemic young rabbit ear wounds, in which it stimulated increased new granulation tissue formation and TGF-β1 expression [140]. In ischemic wounds, no change was found, however, with M-CSF application. This finding suggests adequate oxygen delivery as an absolute requirement for wound healing unable to be superseded by exogenously added factors. MCP-1, which activates and chemoattracts macrophages to the wound bed, shows a modest increase in healing of excisional wounds [24] but less than GM-CSF. In murine wounds, IL-8 and GRO-α stimulate a modest increase in reepithelialization [26,141].

The potential role of TGF-β as a therapeutic agent is less clear. In vitro studies showed the crucial role TGF-β plays in chemotaxis, cellular proliferation, and matrix remodeling; however, its elevated levels also have been shown to be associated with pathologic fibrosis from hypertrophic scar formation to cirrhosis. Despite this, animal research shows that TGF-β1 and TGF-β2 can accelerate the repair of incisional and excisional wounds [142–144]. TGF-β3 also has shown ability to accelerate open wound closure without alteration of scar prominence [145]. In particular, a surgical repair study showed that TGF-β eliminated the incidence of incisional hernias in a rodent model [16]. Enthusiasm for these results must be tempered because Wu and colleagues [146] showed the absence of wound healing enhancement by TGF-β1 in aged animals. This issue needs to be examined seriously because several reports are being published denoting the pro–wound healing properties of TGF-β isoforms in young animal models, yet most chronic, poorly healing wounds occur in the elderly.

Other single IM application studies have had mixed results in attempting to accelerate wound healing. Pressure ulcers have been treated with IL-1β without enhanced healing [147]. Although IL-2 has shown modest wound improvement in doxorubicin-treated rats [148]. The specific tissue treated also may be important. In IL-1 receptor–deficient mice treated with IFNs, no change in wound healing was found on skin wounds in contrast to significant wound healing enhancement found in oral wounds that were impaired and exhibited a persistent inflammatory infiltrate [149].

**Summary**

This article provides much evidence that the inflammatory process has direct effects on normal and abnormal wound healing. As better
understanding develops for the mechanism for these outcomes, targeted proinflammatory and anti-inflammatory interventions are likely to be successful. When inflammation is maintained as a regulated and orchestrated response, effective and normal wound healing is likely to result.

References


