Numerous inflammatory cytokines and growth factors have been identified and are known to be essential for normal wound healing and host defense, and many have been implicated in disease states treated by plastic surgeons. Cytokines and growth factors are members of a large functional group of polypeptide regulatory molecules secreted by different cell lines. These peptides exert their influence through autocrine and paracrine fashions within sites of injury and repair. Although cytokines and growth factors are crucial in initiating, sustaining, and regulating the postinjury response, these same molecules have been implicated in impaired wound healing, abnormal scarring, and chronic cutaneous diseases. Therapeutic manipulation of inflammatory mediators in normal and impaired wounds has been performed, with mixed clinical results, but evolving strategies such as gene therapy, as well as further characterization of the cellular-mechanism cytokines and growth-factor triggers, will further add to our therapeutic options. This article discusses the current understanding of important cytokines and growth factors involved in the normal injury response and then addresses pathological states associated with an inappropriate expression of these mediators. Finally, a summary of various cytokine and growth factor-directed strategies being used in impaired wound healing states is presented. (Plast. Reconstr. Surg. 108: 719, 2001.)

The plastic surgeon is a master of the art of tissue manipulation, whether to reconstruct the deformed or injured part or to improve on the existing normal structure. In this role, the plastic surgeon depends on a predictable healing response from the tissues that are being rearranged. These tissue rearrangements are viewed teleologically by the body as injury, even if the goal is to restore normality of function and appearance. The human response to injury includes an orchestration of various specific and nonspecific soluble factors and cellular elements. The appropriate response leads to efficient control of infection and repair of injury. An attenuated response may lead to poor healing and/or prolonged disability. Thus it is a balanced and regulated cascade that most predictably leads to recovery.

Many pro-inflammatory and anti-inflammatory cytokines and growth factors have been studied and are essential for wound healing and host defense. Cytokines and growth factors are members of a larger functional group of polypeptide regulatory molecules secreted by many different cell lines, including immune cells and fibroblasts that exert their influence through autocrine and paracrine fashions within sites of injury. Although these peptides are biologically alike, the terms cytokine and growth factor have been used with different implications for their activity. Cytokine is generally used to describe proteins essential for host defense, whereas the term growth factor has been reserved for polypeptides whose primary role is cell maturation. However, the actual activity of these molecules may be similar or identical within any given cascade of events, so that for the purposes of this discussion, the two terms will be used interchangeably.

Although cytokines and growth factors are crucial in initiating, sustaining, and regulating the postinjury response, these same molecules have been implicated in impaired wound healing, scarring, and chronic cutaneous conditions. An understanding of the necessary cytokine and growth-factor response and its dysregulation in disease is important in treating impaired wound healing states. Important to this concept is not only the magnitude of this response but maybe more importantly the temporal and spatial pattern of cytokine and growth-factor expression during normal and impaired wound healing. Thus the question arises as to how this cytokine response might be modulated to achieve the desired effect of recovery under seemingly disparate conditions.
To answer the above question, we will first define the roles of important cytokines and growth factors involved in the initial response to injury. At the same time, we will describe pathological states associated with an inappropriate expression of these mediators. Finally, we will summarize various cytokine- and growth factor–directed strategies being applied to alter the microenvironment seen in impaired wound healing states.

**Cytokines**

Cytokines are small, pleiotropic protein hormones that are secreted by various cell lines in the body but predominantly by immune cells.1 Cytokines are important mediators of host defense and postinjury-repair responses. In addition, many cytokines may act as regulators of cell growth and maturation.1,2 Research into the biology of cytokines continues to elucidate the role and mechanisms by which cytokines direct and/or impair wound-healing processes (Table I).

**Pro-inflammatory Cytokines**

Tumor Necrosis Factor-α

Tumor necrosis factor-α (TNF-α) is released primarily by the macrophage-monocyte lineage of cells and is crucial in initiating the immune cascade during the host response to injury or bacteria. A variety of stimuli, including parasites, tumors, and endotoxins, can stimulate cells to produce TNF-α.3,4 TNF-α is involved in the recruitment and maturation of the cellular component of inflammation. This includes the upregulation of cell-surface adhesion molecules that play an important role in the immune cell to endothelium interaction, which is necessary for neutrophil chemotaxis.2,5

Tissue levels of TNF-α have important local paracrine effects in initiating proper and expedient wound healing.6,7 TNF-α is detectable locally within 12 hours after experimental wounding and its level peaks after 72 hours.8 Its effects include hemostasis, increased vascular permeability, and increased vascular proliferation.2 TNF-α also promotes many cellular metabolic events that contribute to the supply of nutrient substrates and acute-phase protein synthesis essential for wound healing.9

In contrast, an excessive production of TNF-α may lead to adverse clinical outcomes. Excess circulating TNF-α has been associated with multisystem organ failure and increased morbidity and mortality in inflammatory disease states.10–14 This effect is partially mediated through the recruitment and activation of macrophages and neutrophils, the same cells that are essential for wound healing.15,16 The activation of these immune cells may lead to increased tissue damage through the production

<table>
<thead>
<tr>
<th>Cytokine</th>
<th>Cell Source</th>
<th>Biological Activity</th>
</tr>
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<tbody>
<tr>
<td>Pro-inflammatory cytokines</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TNF-α</td>
<td>Macrophages</td>
<td>PMN margination and cytotoxicity, ± collagen synthesis; provides metabolic substrate</td>
</tr>
<tr>
<td>IL-1</td>
<td>Macrophages</td>
<td>Fibroblast and keratinocyte chemotaxis, collagen synthesis</td>
</tr>
<tr>
<td>IL-2</td>
<td>Keratinocytes, T lymphocytes</td>
<td>Increases fibroblast infiltration and metabolism</td>
</tr>
<tr>
<td>IL-6</td>
<td>Macrophages, PMNs</td>
<td>Fibroblast proliferation, hepatic acute-phase protein synthesis</td>
</tr>
<tr>
<td>IL-8</td>
<td>Macrophages, Fibroblasts</td>
<td>Macrophage and PMN chemotaxis, keratinocyte maturation</td>
</tr>
<tr>
<td>IFN-γ</td>
<td>T lymphocytes, Macrophages</td>
<td>Macrophage and PMN activation; retards collagen synthesis and crosslinking; stimulates collagenase activity</td>
</tr>
<tr>
<td>Anti-inflammatory cytokines</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IL-4</td>
<td>T lymphocytes, Basophils, Mast cells</td>
<td>Inhibition of TNF, IL-1, IL-6 production; fibroblast proliferation, collagen synthesis</td>
</tr>
<tr>
<td>IL-10</td>
<td>Macrophages, Keratinocytes</td>
<td>Inhibition of TNF, IL-1, IL-6 production; inhibits macrophage and PMN activation</td>
</tr>
</tbody>
</table>

PMNs, polymorphonuclear cells.
of reactive metabolites, proteolytic enzymes, and arachidonic acid metabolites.\textsuperscript{17,18}

Although TNF-$\alpha$ seems to be essential to the recruitment and activation of inflammatory cells in the early wound, investigators have shown disparate effects in the maturing and/or chronic wound. Recombinant TNF-$\alpha$ applied locally to experimental wounds increased both wound-disruption strength and collagen synthesis in normal and doxorubicin-impaired animals.\textsuperscript{19,20} In addition, TNF-$\alpha$ induced fibroblast ingrowth and incorporation into a collagen-gel matrix in vitro experiments.\textsuperscript{21}

By contrast, most researchers have reported a deleterious effect of TNF-$\alpha$ on wound biology. Specifically, Rapala et al. demonstrated that TNF-$\alpha$ reduced granulation tissue in-growth after 7 days in acute experimental wounds in a dose-dependent fashion, although this effect was not sustained at 14 and 21 days.\textsuperscript{22} The mechanisms by which TNF-$\alpha$ may adversely effect wound healing include decreased collagen synthesis through the decrease of both collagen hydroxyproline and pro-alpha I chain production.\textsuperscript{23,24}

Additionally, TNF-$\alpha$ has also been implicated in contributing to the poor wound healing seen in septic and chronic disease states. Cooney et al. showed that granulation tissue infiltration was impaired during experimental sepsis in rats and was partially reversed through the administration of a TNF-$\alpha$ antagonist.\textsuperscript{25} Similarly, rats with chronically elevated TNF-$\alpha$ levels had impaired wound healing versus controls.\textsuperscript{26} More importantly, recent studies have observed elevated levels of TNF-$\alpha$ in nonhealing versus healing wounds, implicating it in the pathogenesis of poor wound healing.\textsuperscript{28,40} Hence the early beneficial responses of IL-1 in wound healing become maladaptive if elevated levels persist in wounds beyond the first week after injury.

Interleukin-1

Interleukin 1 (IL-1) shares many properties with TNF-$\alpha$. IL-1 is produced primarily by cells of the monocyte-macrophage lineage of cells but also by keratinocytes in active wounds.\textsuperscript{29,30} Similarly to TNF-$\alpha$, IL-1 activates neutrophils, upregulates adhesion molecules, and promotes chemotaxis.\textsuperscript{9} In addition, IL-1 promotes other cells, such as endothelial cells, to secrete pro-inflammatory cytokines, further contributing to local inflammatory processes.\textsuperscript{31} Thus the IL-1 response may be essential to a long-term host defense.\textsuperscript{32} On the contrary, some studies implicate a role for IL-1 in sepsis, cachexia, and chronic disease states.\textsuperscript{33–37}

Like TNF, many effects of IL-1 are important in wound healing. IL-1 levels become detectable within the first 24 hours of experimental wounding, peak between the first and third days, and then rapidly decline throughout the first week.\textsuperscript{30,38} IL-1 has been shown to increase fibroblast and keratinocyte growth as well as collagen synthesis.\textsuperscript{29} Vegesna et al. showed that IL-1 topical application reversed adverse wound healing in irradiated mice.\textsuperscript{39} On the contrary, several studies have demonstrated higher levels of IL-1 in chronic nonhealing wounds than in healing wounds, implicating it in the pathogenesis of poor wound healing.\textsuperscript{28,40} Hence the early beneficial responses of IL-1 in wound healing become maladaptive if elevated levels persist in wounds beyond the first week after injury.

Interleukin-2

Interleukin-2 (IL-2) is an important cytokine produced by T lymphocytes and is involved in T-cell activation. Although lymphocytes represent the last immune cell population to infiltrate wounds, they seem to be essential to sustaining the postinjury response, and IL-2 may play an important role in this process. IL-2 has been shown to increase fibroblast metabolism in vitro models.\textsuperscript{41} More importantly, IL-2 administration promoted the infiltration of inflammatory cells and fibroblasts in the wounds of doxorubicin-impaired rats, resulting in increased wound-breaking strength.\textsuperscript{42} However, no difference from baseline was seen in control rats that received IL-2, thus suggesting that IL-2 may play an important role in the wound biology of immunocompromised hosts.

Interleukin-6

Interleukin-6 (IL-6) is an important pro-inflammatory cytokine, with a wide variety of effects. The most important IL-6 functions include stem-cell growth, B- and T-lymphocyte activation, and the regulation of the synthesis of hepatic acute-phase proteins.\textsuperscript{43–47} These acute-phase proteins are essential to host immune, coagulation, and metabolic responses to injury. IL-6 is released in response to multiple
stimuli, with TNF-α, IL-1, and endotoxins being potent agonists.\textsuperscript{48,49}

IL-6 is synthesized by many different cells in the body, but like other pro-inflammatory cytokines, the cells of the macrophage and monocyte lineages are particularly important sources. Within wounds, IL-6 is also secreted by polymorphonuclear cells (PMNs) and fibroblasts.\textsuperscript{50} In fact, a rise in IL-6 concentration parallels the increase in PMN count locally within acute wounds.\textsuperscript{51} IL-6 is detectable within 12 hours of experimental wounding and may persist at high concentrations for longer than a week.\textsuperscript{30,50}

IL-6 has been shown to be a potent stimulator of fibroblast proliferation, and, importantly, this effect is abrogated through the administration of selective anti–IL-6 antibodies.\textsuperscript{50,52} In addition, IL-6 has been shown to activate endothelial cell–derived phosphatase activity, an important enzyme in the protection from ischemic injury in the early wound.\textsuperscript{53} Interestingly, Goodman and Stein reported that basal and induced release of IL-6 declines with the aging of fibroblasts. This observation suggests that impaired IL-6 secretion may contribute to the impaired wound healing seen in older individuals.\textsuperscript{54} Similarly, IL-6 production is diminished in fetal wounds, whereas the exogenous administration of IL-6 to these wounds has been shown to lead to scarring. Thus the diminished pro-inflammatory cytokine response seen in fetal wounds may explain the scarless wound healing seen in utero and provides evidence that IL-6 may be involved in scar formation in adults.\textsuperscript{55}

Circulating levels of IL-6 are increased in vivo during both acute bacteremia and chronic disease states and is routinely detected in most other inflammatory states.\textsuperscript{32,56–58} In burns and experimental wounds, this rise in plasma IL-6 levels parallels the rise in concentration of IL-6 within the wound.\textsuperscript{30,50} This observation supports the hypothesis that wounds themselves are an important source of systemic IL-6 secretion. The readily detectable plasma IL-6 concentration seen during severe injury has attracted attention to it as a prognostic marker of severity and/or mortality. In fact, higher plasma levels of IL-6 plasma were detected in nonsurvivors of severe burns versus survivors or controls.\textsuperscript{59,60}

**Interleukin 8**

Interleukin 8 (IL-8) and other members of the chemokine family are emerging as important pro-inflammatory cytokines in injury.\textsuperscript{61} IL-8 is secreted primarily by macrophages but also by fibroblasts in the acute wound.\textsuperscript{5,62} Major IL-8 effects include increased neutrophil and monocyte chemotaxis, increased neutrophil degranulation, and increased expression of endothelial cell adhesion molecules.\textsuperscript{63} IL-8 is found maximally within the first day of injury and seems to be important in promoting keratinocyte maturation and margination.\textsuperscript{64,65} Recent experiments have demonstrated that low-energy cutaneous laser irradiation increases local IL-8 levels in a dose-dependent fashion and suggests that the laser’s beneficial effect on enhanced wound healing may be partially mediated through IL-8 production.\textsuperscript{66}

On the contrary, IL-8 is secreted in higher concentrations by fibroblasts of patients with psoriasis versus normal controls, hence implicating it in the pathogenesis of the psoriatic wound-healing phenotype.\textsuperscript{67} In addition, Liechty et al. found a remarkable paucity of IL-8 mRNA expression in the fibroblasts of fetal wounds versus those of adult controls and concluded that such a diminished pro-inflammatory response in fetal tissue may contribute to the scarless wound repair seen in utero.\textsuperscript{62} Thus, although IL-8 plays an important role in early keratinocyte maturation, in excess its effects may be quite detrimental to wound healing and scar formation.

**Interferon-γ**

Interferon-gamma (IFN-γ) is an important cytokine produced primarily by T lymphocytes and macrophages.\textsuperscript{68} It predominant effects include macrophage and PMN activation and increased cytotoxicity.\textsuperscript{68} IFN-γ plays an important role in the tissue remodeling of wounds. In addition, IFN-γ has been shown to locally reduce wound contraction. These effects are mediated by retarding collagen production and lattice crosslinking while collagenase production increases.\textsuperscript{69–71} These properties have stimulated much attention in IFN-γ therapy for the treatment of hypertrophic and keloid scars.\textsuperscript{72} On the other hand, IFN-γ has been shown to impair reepithelialization and wound disruption strength in a dose-dependent fashion when applied locally or given systemically in experimental wound models.\textsuperscript{73,74} Combined, these observations suggest that IFN-γ administration can improve scar hypertrophy but that this effect may come at the cost of decreased wound strength.
ANTI-INFLAMMATORY CYTOKINES

Interleukin-4

Interleukin-4 (IL-4) is a potent cytokine produced primarily by T cells but also by mast cells and basophils.\textsuperscript{75,76} The most prominent effects of IL-4 include B-lymphocyte proliferation, immunoglobulin E (IgE) antibody-mediated immunity, and inhibition of the macrophage production of pro-inflammatory cytokines (TNF-α, IL-1, and IL-6).\textsuperscript{77} It is this latter effect that makes IL-4 an important anti-inflammatory, counterregulatory cytokine. Within wounds, IL-4 promotes fibroblast proliferation and collagen synthesis.\textsuperscript{78,79} More importantly, IL-4 may direct and stimulate proteoglycan synthesis by wound fibroblasts.\textsuperscript{80} However, IL-4 is found in high skin concentrations in patients with scleroderma and has been implicated in the direction of the fibrotic processes seen in this condition.\textsuperscript{81}

Interleukin-10

Interleukin-10 (IL-10) is an important anti-inflammatory cytokine secreted by macrophages and T lymphocytes. IL-10 mediates its effect through the inhibition of gene expression and synthesis of the major pro-inflammatory cytokines (TNF-α, IL-1, and IL-6).\textsuperscript{68,82} Within wounds, IL-10 levels peak within the first day of injury and are detectable for 10 days afterward.\textsuperscript{83} Through the study of neutralizing antibodies, Sato et al. found that IL-10 inhibits PMN and macrophage infiltration and pro-inflammatory cytokine expression.\textsuperscript{83} Thus, in the acute wound, IL-10 seems to be an important counterregulatory cytokine. However, a sustained anti-inflammatory IL-10 response may also have detrimental effects. In fact, IL-10 is found in extremely high concentrations in chronic venous insufficiency ulcers versus control wounds and has been implicated in contributing to the failure of these wounds to close.\textsuperscript{84}

GROWTH FACTORS

Growth factors and cytokines are both members of a larger group of polypeptide regulatory molecules released by many cell lines in the body. In fact, although they have often been described separately from cytokines, growth factors are cytokines whose primary role is directing the maturation of cells during normal turnover and in the postinjury tissue repair response.\textsuperscript{1,85} Over the past two decades, much research has been conducted in characterizing the role and potential treatment applications of individual growth factors in impaired wound healing states (Table II).

<table>
<thead>
<tr>
<th>Growth Factor</th>
<th>Cell Source</th>
<th>Biological Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>PDGF</td>
<td>Platelets</td>
<td>Activates immune cells and fibroblasts; collagen and proteoglycan synthesis</td>
</tr>
<tr>
<td></td>
<td>Macrophages</td>
<td>Fibroblast chemotaxis and activation; extracellular matrix synthesis</td>
</tr>
<tr>
<td></td>
<td>Keratinocytes</td>
<td>Decreases collagen and fibronectin deposition; reduces scarring</td>
</tr>
<tr>
<td>TGF-β₁ and TGF-β₂</td>
<td>Platelets</td>
<td>Keratinocyte proliferation and migration</td>
</tr>
<tr>
<td></td>
<td>Macrophages</td>
<td>Keratinocyte proliferation and migration; stimulates collagenase activity</td>
</tr>
<tr>
<td></td>
<td>Keratinocytes</td>
<td>Angiogenesis</td>
</tr>
<tr>
<td></td>
<td>Fibroblasts</td>
<td>Endothelial-cell activation and angiogenesis; keratinocyte proliferation and migration</td>
</tr>
<tr>
<td>FGF-1 and FGF-2</td>
<td>Macrophages</td>
<td>Keratinocyte proliferation and migration</td>
</tr>
<tr>
<td></td>
<td>Endothelial cells</td>
<td>Keratinocyte proliferation and migration; stimulates collagenase activity</td>
</tr>
<tr>
<td></td>
<td>Fibroblasts</td>
<td>Angiogenesis</td>
</tr>
<tr>
<td>KGF-1 and KGF-2</td>
<td>Fibroblasts</td>
<td>Keratinocyte proliferation and migration</td>
</tr>
<tr>
<td></td>
<td>Keratinocytes</td>
<td>Keratinocyte proliferation and migration; stimulates collagenase activity</td>
</tr>
<tr>
<td>EGF</td>
<td>Endothelial cells</td>
<td>Angiogenesis</td>
</tr>
<tr>
<td></td>
<td>Keratinocytes</td>
<td>Keratinocyte proliferation and migration</td>
</tr>
<tr>
<td>VEGF</td>
<td>Keratinocytes</td>
<td>Angiogenesis</td>
</tr>
<tr>
<td></td>
<td>Macrophages</td>
<td>Keratinocyte and fibroblast proliferation; collagen synthesis; cell metabolism; endothelial-cell activation and angiogenesis</td>
</tr>
<tr>
<td></td>
<td>Fibroblasts</td>
<td>Keratinocyte and fibroblast proliferation; collagen synthesis; cell metabolism; endothelial-cell activation and angiogenesis</td>
</tr>
<tr>
<td>IGF-1 and IGF-2</td>
<td>Liver</td>
<td>Keratinocyte and fibroblast proliferation; collagen synthesis; cell metabolism; endothelial-cell activation and angiogenesis</td>
</tr>
</tbody>
</table>
Platelet-Derived Growth Factor

Platelet-derived growth factor (PDGF) is essential in initiating and sustaining the wound-healing response. PDGF is released from platelet alpha granules soon after injury. Its immediate effects include the recruitment and activation of immune cells and fibroblasts. Thereafter, PDGF is also secreted by macrophages and stimulates collagen and proteoglycan synthesis. PDGF naturally occurs as three isomers—PDGF-AA, PDGF-AB, and PDGF-BB—each of which promotes wound healing to varying degrees. The three isomers are named according to the arrangement of two polypeptide chains, A or B, which share 60 percent homology and are held together by a disulfide bond. These isomers exert their influence by binding to either of two receptors, α and β, which may only interact with the corresponding A or B chain.

Pierce et al. reported that all three PDGF isomers are found in extremely low levels in both normal skin and chronic nonhealing ulcers. However, PDGF-AA was elevated in both acute wounds or in chronic wounds treated with PDGF-BB. Similarly, levels of PDGF receptors were found to be decreased during impaired wound-healing states. Such observations have led to widespread clinical applications of PDGF. The PDGF-BB isomer is the most widely clinically studied. Specifically, TGF-β1, which may only interact with the corresponding A or B chain.

Transforming Growth Factor-β

Transforming growth factor-β (TGF-β) is released by platelets, macrophages, and fibroblasts within wounds. TGF-β exists as at least three isomers—TGF-β1, β2, and β3—whose most important effects include fibroblast migration, maturation, and extracellular matrix synthesis. Although all three isomers are similar in function in vitro assays, emerging evidence suggests that their biological in vivo activities may be quite different. All three TGF-β isomers induce extracellular matrix deposition by promoting collagen and proteoglycan synthesis while inhibiting protease activity. TGF-β isomers exert their influence through three receptors, which are referred to as types I, II, and III. The type I receptor seems to modulate extracellular matrix synthesis, whereas the type II receptor plays an important role in cell growth. The type III receptor presents biologically active TGF-β to the other two receptor complexes.

These effects make TGF-β important in wound healing and have prompted much research. Of the three isomers, TGF-β1 has been the most widely studied. Specifically, TGF-β1 seems to play an important role in the collagen metabolism and healing of gastrointestinal injuries and anastomoses. In addition, TGF-β1 accelerated wound healing in normal, steroid-impaired, and irradiated animals in experimental wound models.

By contrast, the potent fibroblast mitogenic effects of TGF-β have been implicated in fibrogenesis. Several fibroproliferative disease states, such as scleroderma and interstitial pulmonary fibrosis, are associated with elevated levels of TGF-β. In terms of impaired wound healing, enhanced expression of TBG-β1 mRNA is found in both keloid and hypertrophic scars. Furthermore, the administration of TGF-β1 led to wound fibrosis in a rabbit fetal wound-healing model. In fact, growth-factor analysis of fetal wounds demonstrates a lack of TGF-β, suggesting that the scarless repair seen in utero occurs in the absence of TGF-β.

Recent evidence suggests that each of the TGF-β isomers has disparate effects on wound healing. Although TGF-β1 and TGF-β2 promote extracellular matrix production and cutaneous wound scarring, TGF-β3 may in fact prevent scarring. Shah et al., in a series of experiments, demonstrated that both the administration of neutralizing antibodies to TGF-β1 and TGF-β2 and the exogenous administration of TGF-β3 led to a marked reduction in wound scarring versus controls. Similar antifibrotic effects of the anti-TGF-β1 antibody have been demonstrated in kidney and lung models. Cumulatively, these results suggest that TGF-β1 and TGF-β2 play an important role in tissue fibrosis and postinjury scarring; there-
fore, therapies directed against them may be useful in treating fibroproliferative disorders.

**Fibroblast Growth Factor**

The fibroblast growth factor (FGF) family of proteins is an important mediator of wound angiogenesis and epithelialization. Although ten members of the family have been characterized, FGF exists largely as two forms: an acidic (aFGF, or FGF-1) and a basic (bFGF, or FGF-2) isomer. Two additional members, FGF-7 and FGF-10, also known as keratinocyte growth factors 1 and 2, are discussed separately. Both aFGF and bFGF share similar effects and bind to the same FGF receptor; however, bFGF is ten times more potent. Both FGF isomers are released by macrophages and endothelial cells within wounds and stimulate fibroblast and keratinocyte proliferation and migration. In addition, bFGF promotes the endothelial-cell growth and migration that are essential for angiogenesis. Finally, bFGF plays an important role in preventing wound contraction and in collagen remodeling.

Given its profound mitogenic effect on keratinocytes and fibroblasts, recombinant bFGF has been used in animal and human trials during both normal and impaired wound-healing conditions. A single application of recombinant bFGF accelerated the rate of epithelialization by 20 percent in porcine wounds. In addition, the administration of recombinant bFGF reversed the impaired wound healing seen in diabetic subjects. Furthermore, recombinant bFGF improved wound healing in ischemic and bacterial-contaminated tissues, suggesting that it may play an important role in the management of locally compromised wounds. In fact, a recently completed large phase III trial in China demonstrated that recombinant bFGF administration accelerated wound-healing times in burns, operative wounds, and chronic dermal ulcers by an average of 3 to 4 days, with a successful closure rate of over 90 percent for all groups.

**Keratinocyte Growth Factor**

Keratinocyte growth factor (KGF) is a member of the FGF family of polypeptides. Two forms, KGF-1 and KGF-2, have been identified and interact with the same receptor. Interestingly, both isomers are secreted by fibroblasts and endothelial cells (both of mesenchymal origin), whereas KGF receptors are found exclusively on epithelial cells of ectodermal origin. Thus KGF may be an important mediator of epidermal-dermal interaction. Both KGF isomers are important regulators of keratinocyte proliferation and maturation. In fact, Werner et al. found that transgenic KGF-1 knockout mice demonstrate epidermal atrophy and abnormal hair follicles. Furthermore, KGF-1 expression is decreased in diabetic and steroid-impaired states. These observations suggest that KGF-1 in particular may be essential in epidermal homeostasis.

The profound mitogenic effect of KGF on epithelialization makes it particularly attractive in modulating wound healing. The administration of recombinant KGF-1 or KGF-2 improved reepithelialization, collagen content, and wound-breaking strength in experimental murine wound models. The later effects of KGF on collagen content and wound-breaking strength are most likely partially mediated through the stimulation of epithelial cells to secrete other growth factors, such as PDGF, TGF, and FGF, because as previously mentioned, KGF does not directly activate fibroblast activity.

**Epidermal Growth Factor**

Epidermal growth factor (EGF) is secreted by keratinocytes and directs epithelialization in an autocrine fashion. In addition, EGF stimulates fibroblast collagenase secretion and thus may be important in wound remodeling. Recent evidence suggests that aged dermal fibroblasts have a decreased EGF-receptor expression and may contribute to the impaired healing seen during aging. In contrast, EGF inhibits fetal wound contraction and thus may contribute to the scarless repair seen in utero.

These effects make the use of EGF attractive in experimental wound-healing models. In fact, several experiments have shown that EGF accelerates wound-healing time and epithelialization in animal burn and wound models. On the contrary, Cohen et al. showed no significant difference in wound-healing times between topically applied EGF and silver sulfadiazine versus silver sulfadiazine alone in a double-blinded, randomized human clinical trial of split-thickness skin wounds.

**Vascular Endothelial Factor**

Vascular endothelial factor (VEGF) is released primarily by keratinocytes but also by macrophages and fibroblasts. VEGF levels
rise steadily after wounding and serve as a potent angiogenic factor. VEGF production is influenced greatly by local tissue conditions, including hypoxia, and also by nitric oxide production. VEGF administration improved granulation-tissue formation in both normal and hypoxic tissues during experimental wounding.

**Insulin-like Growth Factor**

Insulin-like growth factor (IGF)—also known as somatomedin—exists as two isoforms, IGF-I and IGF-II, which help regulate cell metabolism and growth. IGF is produced primarily in the liver and skeletal muscle but is also secreted by fibroblasts, neutrophils, and macrophages within wounds. IGF is largely bound to a carrier protein, which seems to play a major role in regulating its effects. These primary effects of IGF include stimulating fibroblast and keratinocyte proliferation, as well as collagen synthesis. In addition, IGF influences endothelial-cell turnover and may promote angiogenesis. Finally, IGF stimulates the glycogen, protein, and proteoglycan synthesis essential to tissue metabolism.

IGF-I seems to play a greater role than IGF-II in the response to postnatal injury because IGF-II concentrations, although high during fetal life, rapidly decline after birth in most tissues. IGF-I concentrations are normally low in unwounded skin but steadily rise within 24 hours after experimental wounding. Thereafter, elevated levels of IGF may persist locally for several weeks.

By contrast, IGF-I concentration is decreased in the experimental wounds of both diabetic and steroid-impaired animals. More importantly, the exogenous administration of IGF-I improved wound healing in both diabetic and steroid-impaired subjects. Recently, Jeschke et al. successfully transferred the IGF-I gene to cells within thermal injury wounds via nonviral transfection in rats, with a profound improvement in wound-healing rates over controls. These results suggest an important role for IGF in the wound healing of both normal and impaired subjects.

**TREATMENT**

Impaired wound healing can be broadly grouped into two categories: nonhealing or excessive-healing states. As already described, cytokines and growth factors play important roles in the biology and pathology of these disparate states. More excitingly, the application, alteration, and/or neutralization of these mediators in wounds may represent important treatment modalities.

**Nonhealing Wounds**

Nonhealing wounds are chronic open wounds that fail to close and reepithelialize. These wounds include pressure sores, lower-extremity diabetic and venous stasis ulcers, and wounds in immunocompromised subjects. The latter group comprises patients with uncontrolled diabetes mellitus, chronic steroid use, sepsis, and those undergoing systemic chemotherapy and/or radiation therapy. Although many reasons for why these wounds fail to heal have been proposed, no unifying theory exists, and the cause is most likely multifactorial.

Pressure sores are best treated with prevention and alteration in the local forces associated with the wound breakdown and local debridement, when indicated. However, recent clinical trials with recombinant PDGF-BB have shown promising results in accelerating wound-healing rates of advanced pressure sores. By contrast, IGF-I concentration is decreased in the experimental wounds of both diabetic and steroid-impaired animals. Chronic diabetic ulcers secondary to large-vessel occlusive disease often necessitate surgery. On the other hand, lower-extremity ulcers secondary to diabetic microvascular, neuropathic disease and/or venous stasis are often not amenable to revascularization. Once again, altering local factors, including excess pressure, regular surveillance, and meticulous foot care, are tantamount in preventing wound progression. However, the local milieu of cytokines and growth factors may greatly influence wound healing in these patients. KGF-1 and IGF-I levels in particular are diminished in diabetic wounds.

Overall, studies in nonhealing chronic ulcers as a group versus controls have shown extremely low levels of PDGF, whereas the levels of TNF-α, IL-1, and IL-10 were elevated (Fig. 1). The administration of several growth factors, including recombinant PDGF isomers and bFGF, has shown clinical improvement in wound-healing rates in both animals and humans. In fact, becaplermin (Regranex, Ortho-McNeil Pharmaceutical, Inc., Raritan, N.J.), a recombinant human PDGF-BB, is the first FDA-approved growth fac-
tor available for the treatment of diabetic neuropathic ulcers. A multicenter, double-blinded, placebo-controlled trial showed a 48 percent complete closure rate in a PDGF-BB–treated group, versus 25 percent in the control group of locally debrided and adequately oxygenated diabetic neuropathic ulcers. These promising results suggest that cytokine- and growth factor–directed therapies might be successfully applied to other nonhealing wound states.

Patients with overwhelming infection or who are in a state of profound sepsis also demonstrate impaired wound healing. As elucidated earlier, septic states are associated with a robust pro-inflammatory cytokine response that delays wound healing. In these septic patients, the neutralization of the pro-inflammatory cytokines and growth factors that may be responsible for delayed and impaired wound healing offers an attractive means for therapy. For example, the administration of an anti-TNF antibody improved wound healing in experimental sepsis in rats.

Excessively Healing Wounds

Fibrosis and excessive scarring are the hallmarks of many systemic diseases. In the cutaneous injury response, scarring contributes to poor aesthetics and potential loss of function. Cytokines and growth factors play an important role in the pathology of scarring and fibrosis. For instance, as previously mentioned, IL-4 levels are elevated in scleroderma, IL-8 levels are elevated in psoriasis, and TGF-β levels are elevated in scleroderma and interstitial pulmonary fibrosis.

Regarding cutaneous injury, the end result of an excessive or robust wound-healing response includes hypertrophic scars, keloids, and contractures. Both hypertrophic scars and keloids are fibroproliferative states that are often mentioned together but that are distinct entities. By definition, hypertrophic scars are raised but do not overgrow the original wound edges, whereas keloids grow beyond the wound edges. Furthermore, hypertrophic scars often regress with time, whereas keloids may continue to grow. Finally, these two processes occur under different conditions. Hypertrophic scars tend to form when excessive tensile forces are applied to wounds. Keloids primarily occur in dark-pigmented individuals who are predisposed toward them.

Both conditions are associated with an increased, prolonged inflammatory response and extracellular-matrix production, including increased collagen deposition and decreased collagen metabolism and turnover. Cytokines and growth factors likely play a pivotal role in the pathology of keloids and hypertrophic scars. TGF-β1 in particular seems to have the most profound effect on promoting net collagen synthesis favoring fibrosis, and elevated levels of TGF-β1 mRNA have been detected in both keloids and hypertrophic scars.

TGF-β1 and TGF-β2 promote scarring, whereas TGF-β3 may reduce scarring. Both the administration of antibodies aimed at neutralizing the effects of TGF-β1 and TGF-β2 and the
administration of exogenous TGF-β1 have been used to prevent scarring in rats. By contrast, IFN-γ retards collagen production and crosslinking while increasing collagenase activity. These effects also make IFN-γ an attractive agent in the future treatment of hyperproliferative scars.

Wound contractures are the pathologic expression of normal wound contraction, which is essential to wound healing by reducing the area of injury and accelerating wound closure. Wound contractures often occur after burns or injuries occur over areas of movement such as joints, leading to functional loss. Several cytokines and growth factors contribute to wound contraction seen in adults. In contrast, fetal mammalian wound healing occurs with a remarkable absence of wound contraction and scar formation. IL-6, IL-8, and TGF-β are virtually absent in fetal wounds. In fact, the experimental administration of IL-6 and TGF-β promoted scarring and fibrosis in utero. In contrast, bFGF and EGF in a dose-dependent fashion prevent wound contraction in fetal animal wounds. Therapy directed at altering the local concentrations of cytokines and growth factors may prove to be effective in preventing wound contracture, but this must occur without impairing wound strength. Larger clinical trials will be required to assess the efficacy and safety of this strategy.

 Future Applications and Pitfalls

The early optimism for cytokine- and growth factor–directed therapy was followed by mixed clinical results. As we continue to characterize these molecules, our crucial understanding of the cellular mechanisms triggered by them grows. Given the redundant and synergistic effects of these mediators, therapy directed at any one cytokine or growth factor may not attenuate pathological wound-healing responses. In fact, it can be argued, and has been demonstrated, that administration of these proteins themselves may actually impair normal wound-healing responses, and thus our definition of a pathologic wound must be strict.

The timing of these strategies is essential to effective cytokine and growth-factor therapy. Cytokines in particular exert their influence early in the inflammatory phase of wound healing, and thus therapies directed against them must optimally occur early after injury. On the contrary, any adverse cellular events triggered by cytokines and growth factors may continue long after they become undetectable. In such situations, the simple neutralization of these factors alone is not enough. Instead, opposing and antagonizing cytokines and growth factors added to the local milieu may be necessary to initiate a compensatory response.

Finally, the further development of delivery systems to administer these factors into wounds may be required. From local topical application to systemic therapy, these cytokines and growth factors must be sufficiently bioavailable to exert their effects in a timely and appropriate fashion. Topical preparations in particular may have unreliable absorption and utilization by their targets. On the other hand, systemic administration of these factors may have profound unwanted global responses.

Such shortcomings have led to experimentation with targeted gene therapy. Research into gene therapy has led to the successful transfection of local wound fibroblasts with both growth factor and nongrowth factor genes. Several strategies, including viral, plasmid, and liposome systems, have been devised to successfully deliver these gene products. Specifically, PDGF-B, aFGF, EGF, and IGF-I genes have been conferred to cells within wounds. In the case of PDGF-B, EGF, and IGF-I, gene-therapy augmentation improved healing in test subjects versus controls in experimental wounds. The coupling of tissue-specific promoter genes with growth-factor genes may further direct therapy to particular cell lines, improving the therapeutic effect while diminishing toxicity.

Thus the key to cytokine- and growth factor–directed therapy is not only understanding the

FIG. 2. Representation of a hyperproliferative scar. TGF-β levels have been shown to be elevated in hyperproliferative scars, whereas IFN-γ, EGF, bFGF, TGF-β1, and anti-TGF-β1 antibodies are potential treatments (Rx).
pathological wound-healing response but also rather controlling it. Further basic science research as well as large clinical trials will be necessary to accomplish this feat.

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REFERENCES

30. Goretsky, M. J., Harriger, M. D., Supp, A. P., Greenhalgh, D. G., and Boyce, S. T. Expression of interleukin-1 alpha, interleukin-6, and basic fibroblast growth factor by cultured skin substitutes before and


48. Fong, Y., Moldawer, L. L., Marano, M., et al. Endo-


97. Border, W. A., and Noble, N. A. Transforming growth


