



The cytokine milieu of diabetic wounds

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Practice Points

- The complex orchestration of events in normal wound healing is guided by the specific temporal and spatial expression of cytokines and chemokines in the wound; however, the exact coordination of these events is not completely understood.
- The expression of cytokines and chemokines is altered in diabetic wounds, which results in persistent inflammation and impaired healing.
- Blocking the activity of individual proinflammatory cytokines (IL-1 β , TNF- α , C-reactive protein) has improved diabetic wound healing in both animal models and humans.
- Diabetic wound healing may also be improved by increasing the expression of anti-inflammatory cytokines (IL-10 and TGF- β).
- The administration of chemokines (or drugs that induce chemokine expression) may improve leukocyte migration and healing in the wound.
- Because of the complexity of wound healing, a multifaceted approach targeting multiple cytokines (thereby altering the cytokine milieu of the diabetic wound) may be most effective for improved healing.

Nonhealing diabetic foot ulcers are a significant complication of diabetes. They develop due to peripheral neuropathy, and may take weeks or months to close. A failure to heal may necessitate lower limb amputation causing significant morbidity and mortality. Wound healing impairment in diabetic patients is associated with delays in immune cell migration and altered macrophage activation. These processes are orchestrated by the cytokine milieu in the wound manifested by an upregulation of proinflammatory cytokines and downregulation of anti-inflammatory cytokines. This review examines the current knowledge of cytokine expression (IL-1 β , IL-6, IL-10, TNF- α , TGF- β , and C-reactive proteins as well as the chemokines CCL2 and CXCL12) and explores potential cytokine immunotherapy to aid healing.

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Background

Diabetes mellitus is a prevalent metabolic disease that is characterized by chronic hyperglycemia and long-term complications such as retinopathy, hypertension and abnormal lipid metabolism [1]. In addition, other complications such as peripheral neuropathy and impaired wound healing lead to chronic foot ulcers, a major cause of diabetes-associated lower limb amputations.

The wound healing process is a complex orchestration of events including inflammation, angiogenesis and extracellular matrix growth and remodeling [2]. The impaired healing observed in diabetic wounds correlates with decreased keratinocyte, fibroblast and immune cell migration into the wound and reduced endothelial cell angiogenesis [3,4]. The movement and function of these cells in the wound are controlled by the local cytokine milieu. In particular, the cytokine milieu plays an important role in differentiating macrophages into subsets that exist on a functional spectrum ranging from proinflammatory (classically activated M1) to anti-inflammatory and healing (alternatively activated M2) macrophages. Diabetic wounds have been linked to both increased proinflammatory cytokine production and an increased ratio of classically to alternatively activated macrophages (M1/M2) [5]. This review focuses on cytokines that are differentially expressed in diabetic wounds, and it highlights research aimed at altering the cytokine milieu in the diabetic wound as an aid to healing.

Interleukins• **IL-1 β**

IL-1 β is an important inflammatory molecule produced by blood monocytes and tissue macrophages [6]. This cytokine is activated via caspase-1 cleavage in the secretory lysosome, after caspase-1 activation by the NALP3 inflammasome. This cycle allows IL-1 β to amplify its own secretion via its initiation of the assembly of the NALP3 inflammasome. Along with activating the inflammasome, IL-1 β also stimulates inflammation via increasing mobilization of leukocytes from the bone marrow and secretion of acute-phase proteins from the liver [7].

Obese patients may have a sustained release of IL-1 β from adipose tissue, and this could have broad effects based on the wide distribution of the IL-1 receptor. Indeed, elevated IL-1 β has been implicated in the development of insulin resistance and aberrant healing in diabetes [8].

Human diabetic foot ulcers have increased levels of IL-1 β , and these levels decrease as the ulcers heal [9]. Using skin explants, topical treatment of IL-1 on normal tissue correlated with increased CXCR2 expression and delayed wound closure [10]. Isolated wound macrophages from diabetic humans and *db/db* mice display increased IL-1 β and NALP3 inflammasome components through 10 days of healing, and blocking of the inflammasome correlated with improved healing in these wounds [11].

Over the past decade, researchers have aimed at blocking the effects of IL-1 β in the hopes of decreasing the chronically inflamed condition of diabetic wounds. Anakinra (Kineret) is an IL-1 receptor antagonist (IL1Ra) commonly used for treating rheumatoid arthritis (RA). Due to the implications of IL-1 β in pancreatic beta cell destruction, much work has been done in using IL1Ra to combat diabetic pathogenesis [12]. Indeed, IL-1Ra and anti-IL-1 β antibody treatments have correlated with improved beta cell functionality in Type 2 diabetic patients, indicating the importance of this cytokine in this disease [13,14]. In wound healing, *Anakinra* was shown to be effective at decreasing IL-6 and TNF- α protein levels in wound tissue during the first 48 h postwounding of normal mice [15]. Similarly, both *db/db* mice injected with IL-1 β -neutralizing antibodies and IL-1R knockout mice display wounds with a decreased M1/M2 ratio, decreased IL-6 and TNF- α gene expression and improved healing [16]. Thus, the blocking of IL-1 β function in diabetic wounds with either neutralizing antibodies or the receptor antagonist may have potential in the improvement of diabetic wound healing.

• **IL-6**

IL-6 is a cytokine secreted by T lymphocytes and macrophages and is critically important in host defense [17]. Adipose tissue, particularly visceral fat, is also a significant source of IL-6 [18]. IL-6 stimulates acute-phase protein release from the liver, production of neutrophils in the bone marrow and supports the proliferation of B lymphocytes. It also influences recruitment of leukocytes through the stimulation of IL-8 and MCP-1 secretion from endothelial cells [19]. As such, this cytokine represents a significant portion of the inflammatory response.

Diabetic insulin resistance and β -cell inflammation are associated with increased levels of IL-6 [20]. Rabbits treated with the toxic glucose

analog alloxan monohydrate display significantly elevated blood glucose, delayed wound healing and significantly higher wound expression levels of IL-6 and its receptor GP130 compared with controls [21]. Hyperglycemia has been shown to significantly elevate IL-6 expression in a dose-dependent manner in macrophages isolated from normal mice, and macrophages isolated from streptozotocin (STZ)-injected and *db/db* mice corroborate this effect [22]. Diabetic patients with foot ulcers displayed significantly higher levels of circulating acute-phase proteins and IL-6 than those without foot ulcers [23]. Both glucose concentration and wound chronicity seem to have strong correlation with the increased IL-6 expression observed in diabetic foot ulcers.

IL-6-deficient mice have impaired macrophage infiltration and delayed wound healing, which was not observed in mice lacking the α -subunit of the IL-6 receptor alone [24]. However, blocking the IL-6 receptor has been shown to successfully decrease inflammation in models of corneal alkali burns, indicating a potential benefit in its use in chronic wounds [25]. This may be explained by a need for some level of IL-6 in the normal wound healing process combined with deleterious effects when IL-6 is overexpressed in chronic wounds. *Tocilizumab* (Actemra) is an anti-IL-6 receptor antibody that has been effective in improving blood glucose levels in patients with Type 2 diabetes [26]. In a study of joint surgeries between RA patients treated with nonbiologic antirheumatic drugs and those treated with *Tocilizumab*, the latter group experienced significant depression of post-operative fever and plasma C-reactive protein (CRP) [27]. This suggests an efficient suppression of the inflammatory effects of IL-6 in humans, but it has yet to be tested in a model of diabetic wound healing.

In addition to antibody treatment of IL-6, natural remedies may represent a potential option for lowering its expression in diabetic wound healing. Curcumin, one of the main ingredients in turmeric, has been shown to significantly decrease circulating plasma levels of IL-6 in STZ-injected mice over 7 weeks [28]. Indeed, use of curcumin-loaded poly (ϵ -caprolactone) nanofibers resulted in significantly lower levels of IL-6 release *in vitro* from lipopolysaccharide-stimulated macrophages and improved wound healing in STZ-injected mice [29]. These results suggest that the reduction in circulating IL-6

levels, whether by antibody-mediated or natural treatments, could be a key tool in the field of diabetic wound healing.

• IL-10

Unlike IL-1 β and IL-6, IL-10 is an anti-inflammatory cytokine. *In vivo*, it is mostly secreted by T helper cells, regulatory T cells, macrophages and dendritic cells [30,31]. The IL-10R is mainly expressed on immune cells, predominantly macrophages, where it will inhibit the release of proinflammatory mediators, suppress antigen presentation and enhance phagocytosis [32]. IL-10R signaling is essential for the generation of anti-inflammatory macrophages that regulate mucosal defense in mice and humans [33]. Interestingly, regulatory T-cell-mediated suppression of T helper 17 (Th17)-induced inflammation is mediated by IL-10 secretion [34]. Due to the correlation between autoimmune diseases and Th17 cells, IL-10 involvement in diabetes with regards to this lymphocyte subset should be investigated.

There is an association between obesity, Type 2 diabetes and low circulating levels of IL-10 [35]. IL-10 protein expression is lower in isolated macrophages from *db/db* mice compared with *db/+* mice over 7 days postwounding [36]. In addition, STZ-injected rats have shown significantly lower protein levels of this cytokine in the tissue through 7 days postwounding compared with controls [37]. Human diabetic foot ulcers have decreased expression of IL-10, particularly in the keratinocytes and endothelial cells at the wound margins [38]. Unlike the high expression of pro-inflammatory cytokines, the expression of this anti-inflammatory cytokine in diabetic wounds is quite low, and its paucity may contribute to the development of chronic nonhealing wounds.

There are several methods available for increasing IL-10 expression. Highly purified eicosapentaenoic acid increased IL-10 expression in monocytes derived from obese patients with dyslipidemia [39]. Recently, the topical application of curcumin on wounds of STZ-injected rats correlated with increased IL-10 mRNA and protein, increased wound contraction and improved granulation tissue formation [40]. Lentiviral-mediated IL-10 overexpression in mice has been shown to correlate with decreased scar formation and reduced pro-inflammatory cytokine production, such as IL-6 and MCP-1 [41]. Another potential gene delivery vector is N-acyl low-molecular weight chitosan.

Nanomicelles of this vector designed to express IL-4 and IL-10 were injected intramuscularly into STZ-injected mice, which resulted in significantly higher serum levels of these cytokines and decreased proinflammatory cytokines [42]. Whether by topical treatments or gene therapy, increasing IL-10 in diabetic wounds suggests an interesting option in improvement of healing.

Noninterleukin cytokines

• TNF- α

TNF- α is a proinflammatory cytokine that stimulates inflammation at low levels and inhibits extracellular matrix synthesis at high levels [43]. While the main source of this cytokine is macrophages, it can also be produced by adipose tissue, neurons, mast cells and lymphocytes [44,45]. In combination with IL-1 β and IL-6, it can stimulate the acute-phase response, act as a potent neutrophil chemoattractant and stimulate the classical activation of macrophages. This latter function occurs through its binding with TNFR2, which activates the MAPK and NF- κ B signaling pathways [46]. *In vitro*, TNF- α also stimulates apoptosis of fibroblasts, keratinocytes and endothelial cells [47,48]. This occurs through its binding with TNFR1, which stimulates downstream mitochondrial cytochrome C release, caspase 9 activation and apoptosome formation. TNF- α frequently opposes the proliferative activity of TGF- β , presumably through the inactivation of Smad 2/3 [49]. While TNFR1 is fairly ubiquitous in distribution, TNFR2 is mainly relegated to immune cells, limiting its proinflammatory effects to leukocytes. Consequently, TNF- α signaling is quite complex and sometimes antagonistic, allowing it to perform many functions in the process of wound healing.

STZ-injected rats displayed significantly higher levels of serum TNF- α by day 4 postwounding. Oral supplementation with camel undenatured whey protein reduced both TNF- α expression in the wound and time to wound closure [50]. Diabetic patients have also shown a significant upregulation in serum TNF- α during high blood glucose events compared with a relatively little change observed in normal patients [51]. In addition, TNF- α has also been observed to be elevated in the serum of obese patients [52]. Acute hyperglycemia appears to trigger a much stronger upregulation of TNF- α in diabetics, lending further credence to the overall chronic inflammatory state seen in this disease.

Anti-TNF- α neutralizing antibodies administered to *ob/ob* mice significantly improved healing and reduced inflammation and the numbers of viable macrophages at the wound site [53]. Etanercept (Enbrel) is a TNF receptor:IgG1 F_c fusion protein that has been shown to significantly decrease TNF- α activity in the wounds of chronic leg ulcers [54]. It has also been effective in *db/db* mouse wounds, where it significantly decreased fibroblast apoptosis and increased new matrix formation [55]. Infliximab (Remicade) is an anti-TNF- α neutralizing antibody treatment that is used to treat autoimmune diseases such as RA and Crohn's disease. It has been found to be successful at healing human chronic leg ulcers, healing 9 of 14 ulcers by more than 75% within just 8 weeks of use [56]. As of yet, Infliximab has not been tested in any model of diabetic wound healing [57].

• TGF- β

TGF- β acts as a chemoattractant for neutrophils and monocytes early in healing, as well as stimulates monocyte-to-macrophage differentiation, proliferation of fibroblasts and subsequent extracellular matrix synthesis later in the process [58]. Upon injury, it is secreted by platelets, keratinocytes, resident macrophages and fibroblasts [59]. As such, TGF- β experiences a biphasic expression during normal wound healing that peaks within a few hours and again at 5 days postinjury [60]. This cytokine elicits its effects via binding, heterodimerization and phosphorylation of its receptor, which then phosphorylates Smad proteins that translocate to the nucleus and regulate expression of target genes by binding to the promoter elements [61].

A reduction in TGF- β expression has been observed in both human wounds and rodent models of wound healing. Human nonhealing venous ulcers have shown suppressed TGF- β signaling via downregulated TGF- β R and attenuation of Smad signaling, and STZ-injected mice have reduced wound tissue expression of TGF- β on day 4 postwounding [62,63]. In a rat model of Type 2 diabetes, decreased TGF- β signaling was associated with delayed wound closure. *In vitro* analysis of isolated diabetic dermal fibroblast demonstrated reduced TGF- β RII signaling and fibroblast migration [64]. Similarly, human diabetic foot ulcers have decreased expression of both TGF- β and its receptor in the wound [65]. Importantly, the concentration of this cytokine in the wounds of Type 2 diabetic patients

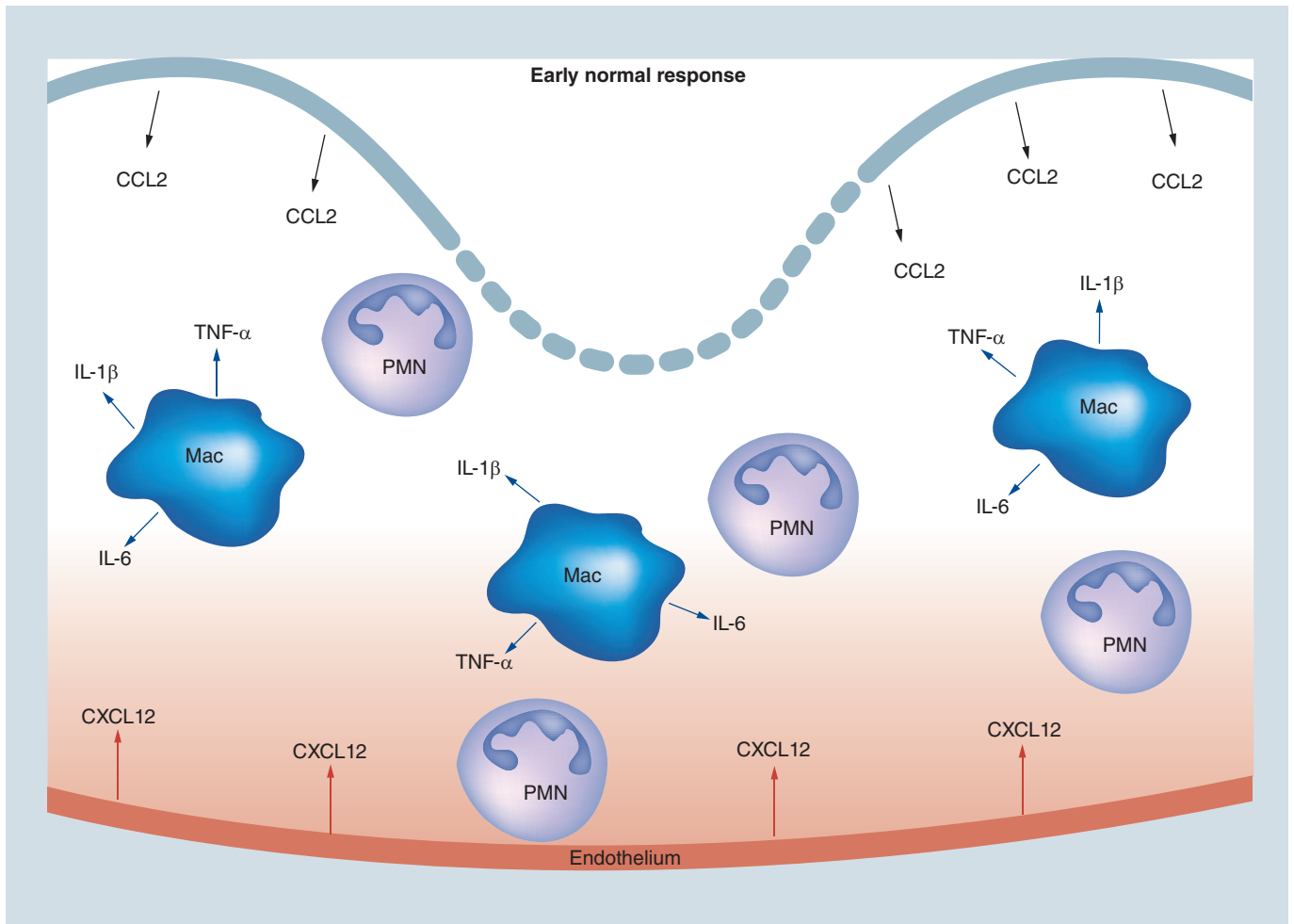


Figure 1. Early events in normal wound healing include the production of chemokines: CCL2 by keratinocytes and CXCL12 by endothelial cells. These molecules recruit polymorphonuclear cells and macrophages into the wound bed. At this stage, the macrophages have an M1 phenotype that is characterized by production of the proinflammatory cytokines IL-1 β , IL-6 and TNF- α .

correlates with decreased levels of matrix metalloproteinases and a concomitant increase of tissue inhibitor of matrix metalloproteinases [66]. As a consequence, the lower concentration of TGF- β found in diabetic wounds may delay wound healing by preventing the growth of the proliferative phase and allowing the disintegration of the extracellular matrix by the matrix metalloproteinases.

Studies have shown that intradermal injection of a TGF- β -expressing plasmid significantly improves wound closure, collagen synthesis and angiogenesis in *db/db* mice [67,68]. In a blinded study of human diabetic neuropathic ulcers, applications of TGF- β correlated with improved healing compared with controls alone [69]. It has been suggested that because the latent form TGF- β binds to the extracellular matrix, this form may be a more effective *in vivo* therapy than

active TGF- β due to its increased half-life [70]. This would indicate that treatment with a drug, such as nitric oxide, that can activate the latent form may be a more appealing alternative. For instance, the application of ointment from the *Momordica charantia* fruit has improved wound closure, increased granulation tissue formation and increased TGF- β detection in STZ-injected rats [71,72]. Accordingly, drugs that could increase endogenous levels of active TGF- β may be more effective than gene therapy approaches.

• C-reactive protein

CRP is a highly conserved acute-phase protein of hepatic origin that acts as a pattern recognition receptor. Structurally, it consists of five identical protomers that each contains a phosphocholine binding site, which together form a pentraxin around a central core. Functionally,

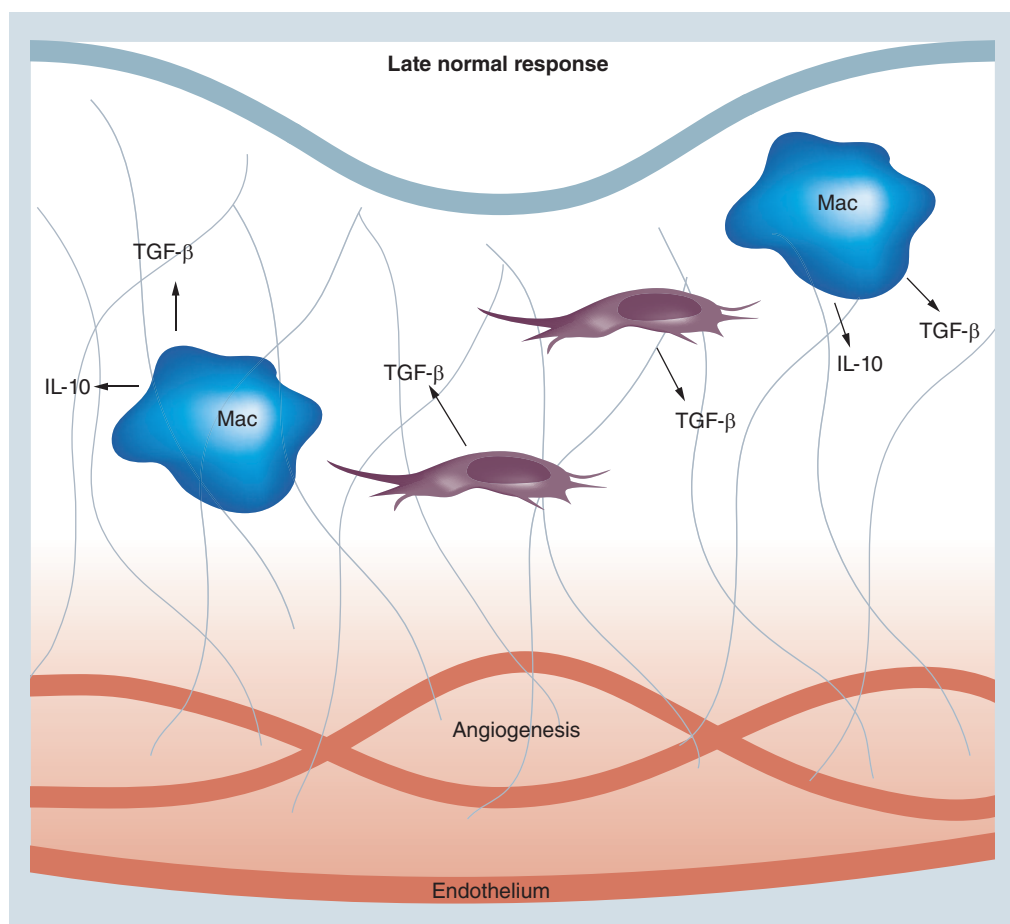


Figure 2. In the later stages of normal wound healing there is reepithelialization, angiogenesis and the rebuilding of extracellular matrix fibers. The macrophages display a more M2 phenotype through the production of the anti-inflammatory cytokines IL-10 and TGF- β . Fibroblasts also produce TGF- β .

it can activate the classical complement system, stimulate phagocytosis and bind to immunoglobulin receptors [73]. This occurs via its binding to phosphocholine expressed on the surface of dying cells and bacteria. It is also able to upregulate adhesion molecules expressed on endothelial cells and increase the release of proinflammatory cytokines such as IL-1 β , IL-6 and TNF- α [74,75]. As CRP is regulated transcriptionally by IL-6 and IL-1 β , it can result in a cyclic amplification of inflammation.

Both Type 1 and Type 2 diabetic patients have significantly elevated CRP in their plasma [76,77]. In addition, Type 1 diabetic patients with microvascular complications have higher CRP levels compared with otherwise healthy Type 1 diabetics [78]. Diabetic humans with foot ulcers also display significantly higher CRP in their serum when compared with wounded nondiabetic patients or diabetics without foot ulcers,

and CRP levels were significantly lower for those diabetic patients with healed ulcers [79]. This indicates the potential of CRP as a clinical biomarker for diabetic foot ulcer healing.

Statin therapy has been known to significantly decrease circulating CRP levels, as well as improve normal wound healing [80,81]. Both injection and topical application of Simvastatin (Zocor) on *db/db* mice have correlated with enhanced angiogenesis and improved healing through the first week postwounding [82,83]. Likewise, Pravastatin (Pravachol) treatment of STZ-injected mice has resulted in significantly higher wound breaking strength, hydroxyproline content and eNOS expression over 10 days postwounding [84]. Finally, Atorvastatin (Lipitor) has improved healing in STZ-injected rats over 14 days postwounding, as well as decreased serum CRP levels and prevented new diabetic foot ulcers in Type 2 diabetic patients [85,86]. In order

to reduce adverse systemic effects from statin treatment, topical application seems to be the optimal delivery route for improved diabetic wound healing.

Chemokines

• CCL2 (MCP-1)

CCL2, or MCP-1, is a CC family chemokine that is secreted by keratinocytes and acts as a chemoattractant for macrophages and T cells [87]. Along with its chemotactic function, this chemokine also stimulates growth factor production from recently emigrated leukocytes, stimulates fibroblast activity via mast cell-derived IL-4 and increases motility of endothelial cells [88]. Within the wound, CCL2 displays the highest expression during the first 24 h postwounding, with levels dropping off after the first week [89].

CCL2-deficient mice exhibit delayed healing, decreased angiogenesis and collagen production and delayed macrophage migration into the wound [90]. *Db/db* murine wounds have a lower expression of CCL2 than controls within 24 h postwounding, but a higher expression of

this chemokine after 13 days [91]. Bone marrow-derived macrophages from *db/db* mice showed a significant decrease in chemotaxis to CCL2 and impaired scratch wound closure compared with controls, despite expressing normal levels of CCR2, the receptor for CCL2 [92]. This suggests that, in addition to expressing lower levels of CCL2, diabetic wounds may also contain immune cells that are less responsive to its signal.

Recently, it was observed that the local injection of CCL2 upon wounding restored its deficient expression within 24 h, promoted re-epithelialization and improved monocyte homing in *db/db* wounds [93]. Interestingly, low-intensity vibrational treatment of *db/db* mice resulted in improved healing, augmented angiogenesis and significantly increased CCL2 wound expression by day 7 postwounding compared with nonvibrated diabetic controls [94]. This type of mechanical treatment has been supported based on improved blood flow, but the exact mechanisms have yet to be elucidated. STZ-injected mice have shown improved wound healing by day 5 postwounding, increased leukocyte

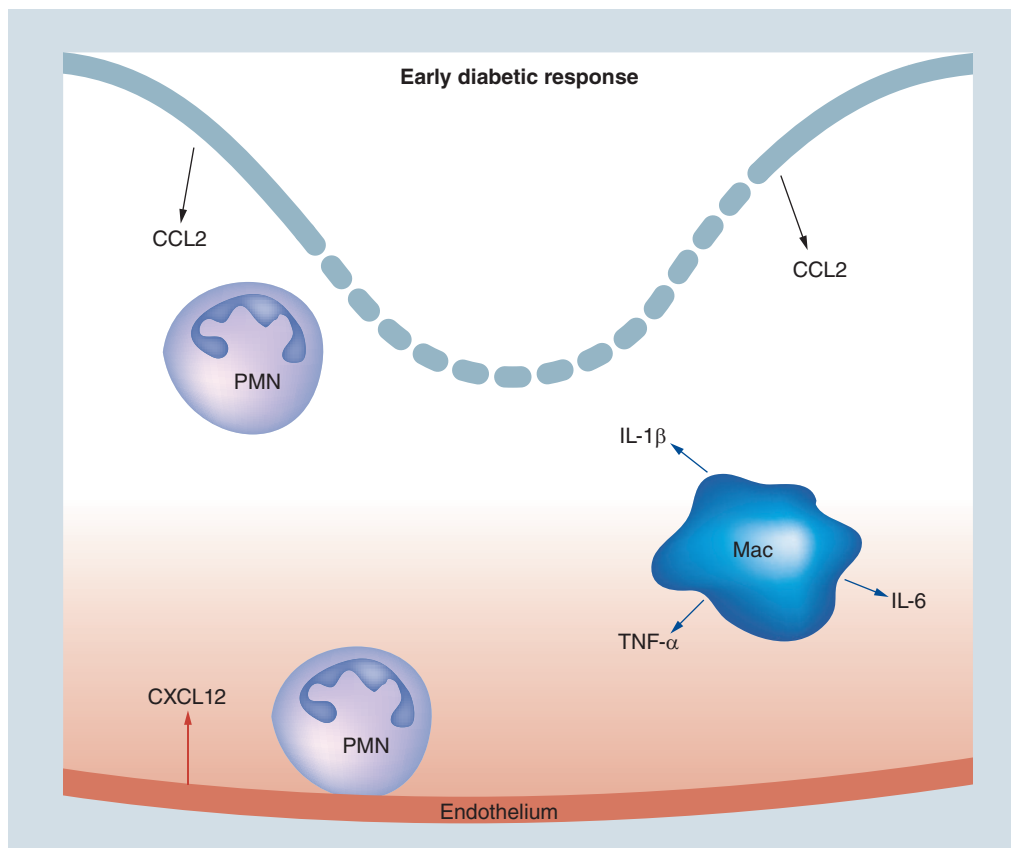


Figure 3. Normal events are impaired in the early stages of diabetic wound healing. Both chemokine production and recruitment of phagocytes to the wound are reduced.

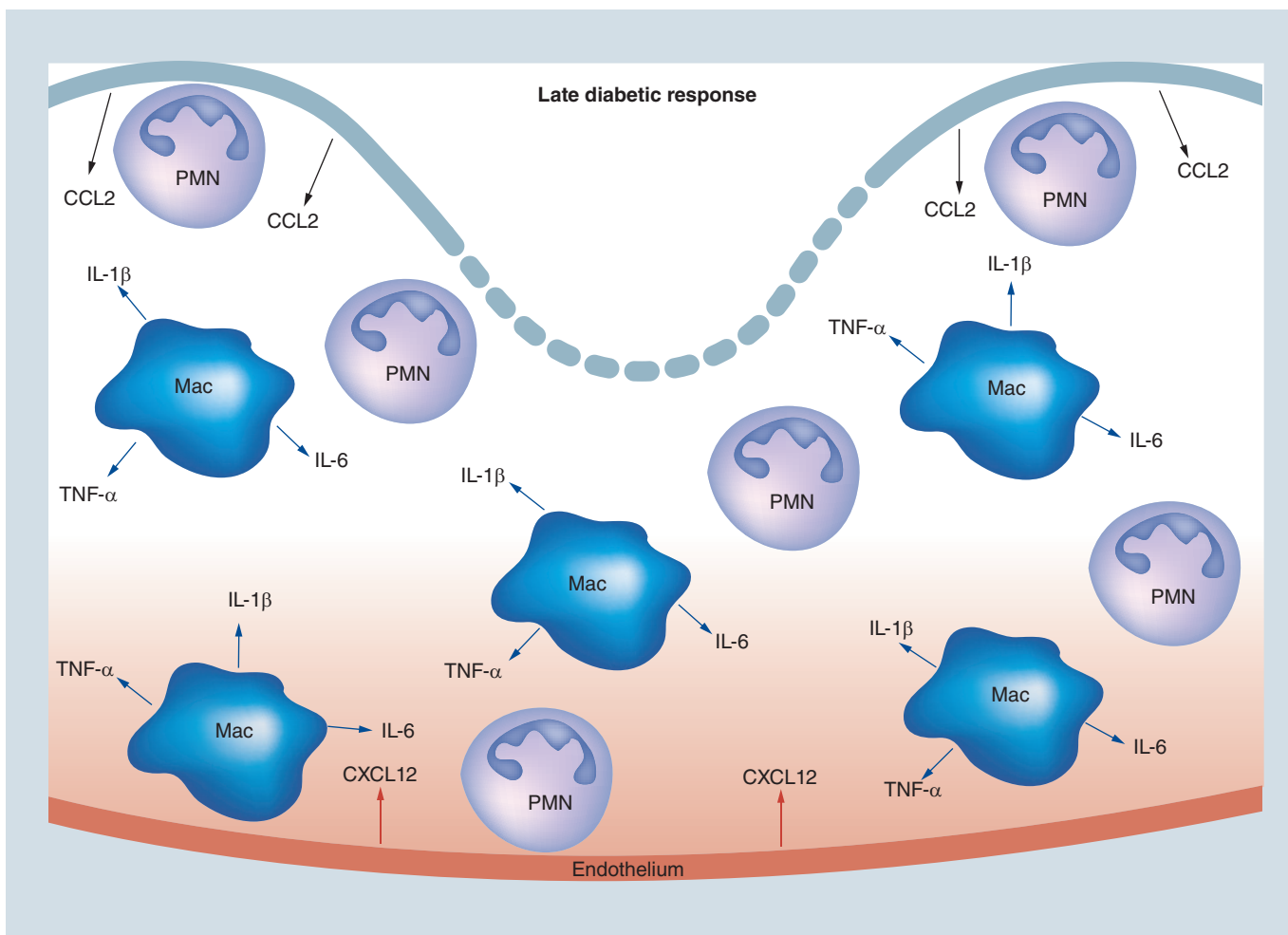


Figure 4. In the later stages of diabetic wound healing, there is persistent production of chemokines (CCL2 and CXCL12) and an associated continued recruitment of neutrophils and macrophages. The macrophages have reduced expression of M2 markers and continue to produce proinflammatory cytokines (IL-1 β , IL-6 and TNF- α).

intravasation and significantly increased CCL2 expression within 24 h in conjunction with local administration of GM-CSF [95]. This study suggests that CCL2 may be an intermediary for the improved leukocyte migration and healing observed in wounds treated with some growth factors.

• **CXCL12 (SDF-1)**

CXCL12, or SDF-1, is a CXC family chemokine expressed by endothelial cells and fibroblasts [96]. Its receptor, CXCR4, is expressed by lymphocytes and monocytes [97]. CXCR4 signaling is involved in trafficking and homing of hematopoietic stem and progenitor cells, as well as tumor metastasis [98]. Importantly, CXCL12 is a potent angiogenic factor that stimulates endothelial migration in a VEGF-independent manner [99]. The recent discovery of CXCR7, a

second receptor for CXCL12, has shed new light on potential additional functions. While not expressed on normal blood leukocytes, CXCR7 has been found on macrophages in pathological conditions. It may be involved in switching macrophages to a proinflammatory phenotype and increasing phagocytic activity [100].

STZ-injected mice show decreased expression of CXCL12 through 9 days postwounding compared with controls, and the injection of reconstituted CXCL12 has improved healing in these wounds [101]. *Db/db* mice also display decreased levels of this chemokine through 7 days postwounding, and the insertion of a plasmid expressing CXCL12 correlated with improved healing [102]. In contrast, the use of a CXCL12 antagonist exacerbated impaired healing in *db/db* wounds. This was evidenced by decreased angiogenesis and granulation tissue

formation, and increased IL-6 and MCP-2 expression [103].

Topical application of carnosine to *db/db* wounds has correlated with increased CXCL12 and improved healing [104]. Lentiviral administration of CXCL12 to *db/db* wounds resulted in increased granulation tissue and improved epithelialization [105]. The use of alginate scaffolds or hydrogel as CXCL12 delivery vehicles improved healing in normal murine wounds [106,107]. These delivery options have, as of yet, to be explored in diabetic wound healing. AMD3100 (Mozobil) is the first CXCR4 antagonist to enter clinical trials and is frequently used to mobilize hematopoietic cells in cancer patients [108]. The disruption of the CXCL12–CXCR4 interaction by this drug increases endothelial progenitor cells in the periphery [109]. Topical application of AMD3100 to *db/db* wounds has caused an increase in CXCL12 expression, improved healing and increased endothelial progenitors in the circulation [110]. However, caution must be exercised when using this drug because it is entirely possible that there are conflicting effects through a second receptor, CXCR7, through which AMD3100 acts as an agonist [111]. Overall, murine diabetic wounds have demonstrated a decreased expression of the CXCL12, which may partially explain the lack of neovascularization observed in these wounds. Several treatment options show promise in reversing this pattern and improving healing.

Conclusion

Wound healing is a complex process and many of the normal wound healing events are impaired in diabetic wounds as illustrated in **Figures 1–4**. In the first hours and days, the initial recruitment of inflammatory cells to the diabetic wound is delayed compared with normal wounds [112]. This is likely due to a reduced or altered chemokine expression, but this mechanism is incompletely understood (**Figure 1 & 3**). At later times, chemokines such as CCL2 and CXCL12 may persist in diabetic wounds [113]. In addition, other studies using *db/db* mice and Type 1 diabetic patients have measured significantly higher levels of other chemokines such as CXCL2 (MIP-2) and CCL5 (RANTES) compared with healthy controls [91,114]. Thus, continued chemokine expression may sustain the presence of proinflammatory cells resulting in a persistent inflamed state of diabetic wounds.

Proinflammatory cytokines in the wound milieu are important in initiating normal

wound repair, but increased expression levels are required only transiently before returning to baseline. Diabetic wounds manifest persistently increased expression of these proteins, resulting in continued inflammatory cell emigration into the wound. This is a major cause of the delay in the healing of diabetic foot ulcers. To date, researchers have identified the proinflammatory molecules IL-1 β , IL-6, TNF- α and CRP to be significantly upregulated and the anti-inflammatory cytokines TGF- β and IL-10 to be significantly downregulated in diabetic wounds (**Figure 2 & 4**). Thus, agents that can block proinflammatory molecules or increase anti-inflammatory cytokines in the wound may be critical to shifting diabetic wounds to a healthy phenotype [114].

Future perspective

This review highlights cytokines and chemokines that are well characterized in the context of diabetic wound healing; however, there is still much that is not known about the intricate dynamics of cytokine expression in both normal and diabetic wound healing. In the future, it will also be important to consider that the cytokine milieu of diabetic wounds may be significantly impacted by the presence of the bacteria and biofilms in the wound. Chronic wounds, including diabetic foot ulcers, have shown higher levels of biofilm than acute nondiabetic wounds; however, only a few studies have examined the diabetic wound microbiome to date [115,116]. *db/db* mouse wounds inoculated with *P.aeruginosa* biofilm displayed higher levels of IL-1 β and IL-6 than control wounds at 4 weeks postwounding [117]. By contrast, in a mouse model of polygenic Type 2 diabetes, wounds inoculated with planktonic *Staphylococcus aureus* unexpectedly had reduced expression of IL-1 β , TNF- α and Toll-like receptors [118]. Thus, future studies should also consider the role of the microbiome in influencing the cytokine milieu and resident cells of the diabetic wounds.

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The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

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