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REVIEW ARTICLE

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# Granulocyte-macrophage colony-stimulating factor: Conductor of the wound healing orchestra?

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**Abstract**

Granulocyte-macrophage colony-stimulating factor (GM-CSF) is a glycoprotein and is derived from both hemopoietic and nonhemopoietic sources which exert immunomodulatory properties. Various theories have been proposed to explain why some wounds become chronic and non-healing. Generalized suppression of inflammation locally or systemically may impede the body's physiological healing response by crippling the activity of reparative cells within the wound ecosystem. Thus, highlighting the importance of promoting host-directed therapeutics with immunomodulatory properties. The temporal and spatial expression of GM-CSF and GM-CSF receptors in the integumentary system suggests that epithelial-derived GM-CSF functions in an autocrine/paracrine manner. This may positively affect wound healing physiology via local inflammatory regulation promoting macrophage survival. Although diabetes negatively affects multiple aspects of wound healing GM-CSF activation is particularly impacted. Compared to acute/healthy wounds diabetic foot ulcers (DFU) only partially activate GM-CSF activity. There is a deleterious chain of events associated with this unfortunate sequala. DFUs also have a high proportion of monocytes and an absence of activated macrophages which results in an impaired inflammatory response. This may potentially serve as a vital point for GM-CSF to act as a companion diagnostic/theragnostic modality to help modulate the inflammatory response in wound healing. Correcting macrophage immune dysfunction with exogenous GM-CSF may help restore the immune balance in the wound ecosystem and jumpstart the wound healing cascade. Thus, the recognized beneficial role of GM-CSF in immune regulation across many studies provides a rationale for the initiation of the ongoing randomized controlled trials using GM-CSF.

**KEYWORDS**

granulocyte-macrophage colony-stimulating factor (GM-CSF), wound care, wound healing

**Key Messages**

- granulocyte-macrophage colony-stimulating factor (GM-CSF) is a glycoprotein and is derived from both hemopoietic and nonhemopoietic sources which exert immunomodulatory properties
- although there is at times a stigma with the term 'inflammation', it is important to keep in mind the inflammatory phase is an essential component of the wound healing cascade; therefore, therapeutic agents should promote immunomodulatory effects rather than simply aim to reduce or eliminate inflammation
- an early and essential effect of GM-CSF is to stimulate the proliferation and migration of keratinocytes and endothelial cells
- studies have revealed that GM-CSF helps facilitate the transition of proinflammatory M1 to pro-healing M2 macrophages
- the temporal and spatial expression of GM-CSF and GM-CSF receptors in the integumentary system suggests that epithelial-derived GM-CSF functions in an autocrine/paracrine manner; this may positively affect wound healing physiology via local inflammatory regulation promoting macrophage survival

## 1 | INTRODUCTION

A wound is a disruption of normal anatomic structure and function that is usually inclusive of the skin. Wound healing is a series of well-orchestrated biochemical events that ultimately lead to tissue regeneration and epithelial contraction which should result in the restoration of anatomical and functional integrity.[1-3] The wound healing process consists of key overlapping phases that include haemostasis, dynamic inflammation, cellular migration/proliferation, protein synthesis and tissue contraction/remodeling.[4] However, on the macro level, there are numerous factors that impact the precise timeline of complete healing which include existing comorbidities, anatomic location, body mass index (obesity),[1] and infection among others. Patients with diabetes are at risk of a plethora of complications including foot ulcers.[5] Diabetic neuropathic ulcers are generally attributed to shear force vectors, unstable lower extremity pathomechanics, and immunologic dysfunction leading to foot ulcerations.[5,6] After a trial of standard-of-care (SOC), wounds that do not heal in a timely fashion may indicate the need to implement advanced wound healing technologies into the treatment algorithm. Granulocyte-macrophage colony-stimulating factor (GM-CSF) is a glycoprotein and is derived from both hemopoietic and nonhemopoietic sources which exert immunomodulatory properties.[7]

## 2 | CURRENT ROUTES OF ADMINISTRATION

Appropriate dose, route and schedules for recombinant human (rhu) GM-CSF in various clinical settings have been defined via, intravenous infusion and subcutaneous injections.[8] In animal models of respiratory infections, the intranasal administration of GM-CSF increased the proliferation of alveolar macrophages and improved outcomes.[9] Given the pleiotropic potential of GM-CSF, host defense and inflammation, care should be taken with respect to dose, route and timing of administration for each therapeutic approach.

## 3 | THE IMMUNOLOGY OF CHRONIC DIABETIC FOOT ULCERS AND THE COMPLEX RELATIONSHIP BETWEEN MACROPHAGES AND GM-CSF

Various theories have been proposed to explain why some wounds become chronic and non-healing. Despite the etiological differences in chronic wounds, they often share common pathophysiologic features which involve interactions of several cell types, extracellular matrix components and regulatory immunologic factors. In chronic wounds, there is markedly reduced cellular division which ultimately impedes cellular growth and proliferation.[10] Transcriptomic RNA-seq data of diabetic foot ulcer (DFU) edges has demonstrated a deregulated immune response with a downregulation of forkhead box protein M1 (FOXM1) FOXM1 and GM-CSF resulting in poor immune cell proliferation and survival.[11] It is important to note that in a chronic wound environment the inflammatory phase of wound healing may either be stalled or prolonged.[1,6] Extended inflammation can be attributed to elevated levels of pro inflammatory cytokines such as TNF-α, IL-6, and IL-1β.[2,3] Vital cells that

promote healing such as fibroblast and keratinocytes are markedly reduced secondary to a proinflammatory state.[4,6] Fibroblasts are critical cells that play a role in the formation of collagen, fibronectin, and other key matrix proteins.[4] Excessive degradation of the extracellular matrix would deprive cells of attachment sites and signals required for migration, differentiation, and proliferation.[12] The resulting wound bed, lacking attachment sites for migration, is "unfriendly" to keratinocytes leading to slow or absent wound closure.[1,13] Many of the biochemical alterations noted in chronic wounds may be responses to lack of adhesion to (or detachment from) an extracellular matrix (ECM) of specific structure and composition at the right time in the wound healing sequence.[12] Cellular senescence within the chronic wound ecosystem can be reversed by modulating the variables that help promote cellular activity.[14]

Although there is at times a stigma with the term 'inflammation', it is important to keep in mind the inflammatory phase is an essential component of the wound healing cascade (Figure 1). Therefore, therapeutic agents should promote immunomodulatory effects rather than simply aim to reduce or eliminate inflammation. As it pertains to pulmonary homeostasis, GM-CSF serves as immunomodulatory function under inflammatory conditions that include infection.[9] Pulmonary alveolar proteinosis (PAP) is a rare syndrome of alveolar surfactant accumulation, resulting in hypoxemic respiratory failure, and increased infection risk commonly categorised as primary, secondary or congenital PAP.[15,16] Primary PAP accounts for the majority of cases and is caused by disruption of GM-CSF signalling, either by GM-CSF autoantibodies (autoimmune PAP)[16] or genetic mutations involving the GM-CSF receptor (ie, hereditary PAP).

Generalised suppression of inflammation locally or systemically may impede the bodies physiological healing response by crippling the activity of reparative cells within the wound ecosystem (Figure 2). Thus, highlighting the importance of promoting therapeutics with immunomodulation rather than complete anti-inflammatory effects. Upon tissue injury, keratinocytes secrete pro-inflammatory cytokines, such as IL-1, TNF-α, and (GM-CSF).[17] GM-CSF mRNA accumulates in keratinocytes early during the course of skin injury.[18] An early and essential effect of GM-CSF is to stimulate the proliferation and migration of keratinocytes and endothelial cells.[18-20] Keratinocytes (in addition to haematopoietic cells) have also been identified as a source and target for GM-CSF.[20] Recruited monocytes are terminally differentiated into macrophages by the local presence of GM-CSF.[21] These early wound healing phase macrophages recognise pathogens and engulf them as well as synthesising metalloproteinases to digest the extracellular matrix and

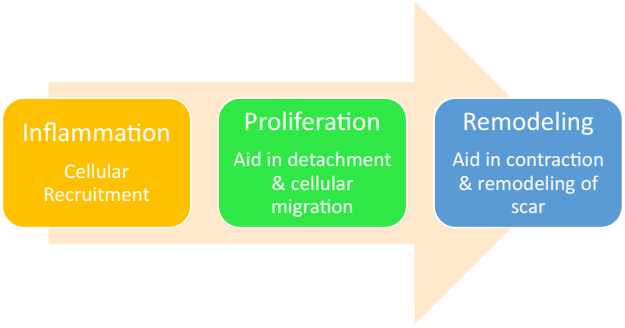


FIGURE 1 Wound healing cascade

the thrombus in-order to facilitate cellular migration.[4] Macrophages require GM-CSF for viability and the bioenergetic activity of their mitochondria essential to their host defence functions and homeostatic activities.[22] Animal studies have demonstrated that GM-CSF overexpression in mice has led to increased re-epithelization and wound closure, whereas GM-CSF depletion impaired wound healing significantly.[7] Keratinocyte-derived cytokines, chemokines, extracellular vesicles, and antimicrobial peptides (AMPs) mediate the dynamic interactions between haematopoietic immune cells and keratinocytes.[23] After the inflammatory phase, the wound enters the growth phase of healing. During this phase, macrophages are transitioning into a pro-healing phenotype.[4] This phenotype begins the process of efferocytosis, the engulfing of apoptotic cells.

Neutrophils play important roles in influencing the behaviour and function of neighbouring cell types during inflammation.[24] Macrophages are programmed by their tissue environment to silently clear apoptotic cells.[25] Apoptotic cells secrete 'find me' signals that induce phagocytic recognition. The apoptotic cell is engulfed by the macrophage this process termed efferocytosis may lead to the successful resolution of inflammation.[26] During this process, the macrophage is filled with a metabolite load almost equal to the size of the cell itself elevating macrophage fatty acid and oxygen consumption.[27] Fatty acid oxidation is essential to macrophage efferocytosis.[27] For this GM-CSF signalling is required.[22] Fatty acid β-oxidation promotes catabolism within the mitochondrial ecosystem.[27]

The dynamic balance between M1-M2 (macrophages) is critical for proper wound healing.[28] In chronic non-healing wounds, failure of macrophages to transition from the pro-inflammatory M1 to a pro-healing reparative M2 phenotype can lead to (Figure 3)[11,28]

- prolonged inflammation
- reduced growth factors
- reduced granulation tissue
- impaired overall healing

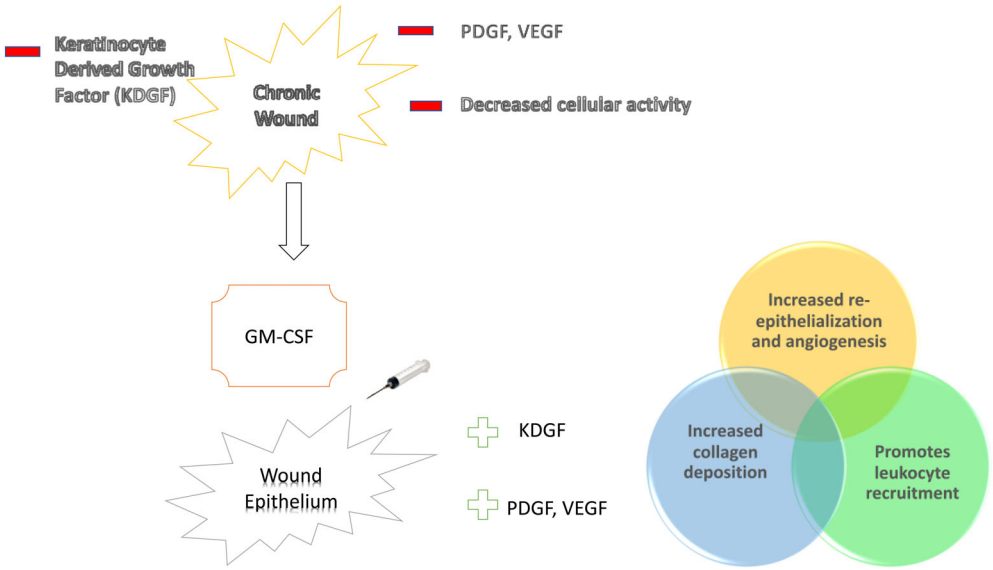


FIGURE 2 Granulocyte-macrophage colony-stimulating factor's role in wound healing

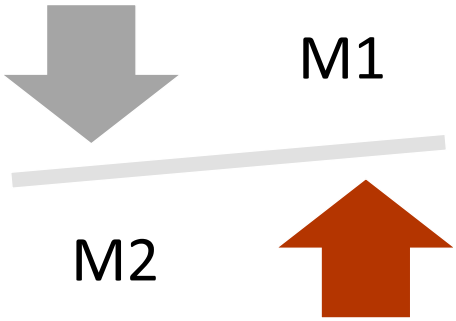


FIGURE 3 Chronic wound M1 and M2 environment


FIGURE 4 Granulocyte-macrophage colony-stimulating factor M1, M2 regulation

Studies have revealed that GM-CSF induces peroxisome proliferator-activated receptors (PPARs) gamma expression. This transcription factor is responsible for catalysing a series of biochemical events that help facilitate the transition of proinflammatory M1 to pro-healing M2 macrophages[29] (Figure 4). GM-CSF fosters adipose MSC regeneration capacity to promote cellular differentiation and tissue repair.[30] Additionally, GM-CSF signals these elevated MSC populations that can be utilised to recruited to sites of tissue injury to promote angiogenesis and eventually differentiate into keratinocytes.[31]

Cianfarani et al demonstrated that GM-CSF injections to non-healing venous leg ulcers stimulated VEGF transcription in the wound bed, primarily within macrophages.[32] VEGF is an important cytokine that is vital in recruiting endothelial cells to the site of injury thereby promoting angiogenesis.[33] Neovascularization of the wound bed is critical in order to receive and optimise essential nutrients for tissue repair.[33] The consequence of a hyperglycemic microenvironment leads to endothelial cell dysfunction, reduced nitric oxide production, increased platelet aggregation, and decreased GM-CSF production.[34,35] The reduced concentration of GM-CSF also contributes to a delay in wound closure. Keratinocytes and fibroblasts are directly affected by GM-CSF levels. As a result of lower levels of GM-CSF, keratinocytes and fibroblasts may not proliferate and migrate towards the site of injury to assist in wound closure. There is a deleterious chain of events associated with this. Key transcription factors such as STAT3 and FOXM1 are only partially activated in DFUs.[11] Phosphorylation of STAT3 is dependent on GM-CSF stimulation.[36] DFUs also have a high proportion of monocytes and an absence of activated macrophages which results in an impaired inflammatory response.[11]

The temporal and spatial expression of GM-CSF and GM-CSF receptors in the integumentary system suggests that intrinsically-derived GM-CSF functions in an autocrine/paracrine manner.[7] This may positively affect wound healing physiology via local inflammatory regulation promoting macrophage survival. Correcting macrophage immune dysfunction with exogenous GM-CSF may help restore the immune balance in the wound ecosystem and jumpstart the wound healing cascade. Thus, the recognising pathogenic role of GM-CSF in immune overactivation

across many studies provides a rationale for the initiation of the ongoing randomised controlled trials using GM-CSF. Understanding the clinical and physiologic cues of wound healing helps pave the way for companion diagnostics/theragnostics. This will help clinicians make critical patient-centered decisions.

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## CONFLICT OF INTEREST

Dr David Armstrong is a consultant for Partner Therapeutics, Inc.

## DATA AVAILABILITY STATEMENT

Data sharing not applicable to this article as no datasets were generated or analysed during the current study.

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