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Neutrophils and natural killer T cells as negative regulators of wound healing

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Cutaneous wound healing is a dynamic process requiring coordination of immune cell infiltration, keratinocyte proliferation, angiogenesis, extracellular matrix deposition and remodeling to ensure adequate wound closure [1–3]. Multiple pathologic conditions are known to perturb efficient cutaneous repair by impacting one or more of the three interdependent phases of wound healing: the early inflammatory, proliferative, or late remodeling phase [1,2,4,5]. Innate immune cells are critical during wound repair, as innate immune cell dysfunction has been demonstrated to augment normal wound healing, leading to poor wound closure or hypertrophic scar formation. While several immune cells, in particular macrophages, dendritic epidermal T cells (DETCs) and mast cells, have all been demonstrated to enhance aseptic wound healing [6–15], two members of the innate immune population, neutrophils and natural killer T (NKT) cells, act to negatively regulate the pace of wound closure. Herein, we focus our discussion on the negative regulatory role of these neutrophils and NKT cells, and consider potential therapeutic interventions that may enhance wound repair by alterations in the number or function of these populations.

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Controversial role of neutrophils in wound repair

Neutrophils are described as the first class of infiltrating immune cells at the site of injury, helping to control infection and remove debris. Despite this necessary function, excessive release of enzymes by neutrophils, such as elastase, can be damaging to surrounding tissue, delaying rates of healing and increasing the risk of scar formation [16–19]. For these reasons, the need for neutrophil infiltration following injury is heavily debated. Supporting an inhibitory effect of neutrophils on wound repair, *in vivo* neutrophil depletion in mice with rabbit anti-mouse sera prior to injury accelerated re-epithelialization through day 3 post injury [18]. These results by Dovi *et al.* are supported by earlier findings that conclude that neutrophils are either dispensable in cutaneous repair [20] or that their excessive presence is detrimental to the healing process [21]. In the later study, impaired healing in a murine model of diabetes was associated with enhanced macrophage inflammatory protein-2 production and a concomitant protracted infiltration of neutrophils at the site of injury [21]. Together, these data suggest that neutrophils may be deleterious by delaying the resolution of the proinflammatory phase and preventing progression into the proliferative phase of wound repair.

Extending the argument for a destructive role of neutrophil recruitment to sites of aseptic injury are fetal wound-healing models. Fetal wound healing is characterized by a markedly reduced inflammatory response, including limited neutrophil recruitment at injury sites, swifter rates of wound closure and minimal, if any, scarring [4,19]. It is often suggested that attempting to mimic these early fetal conditions might enhance wound closure. However, one must consider how the aseptic intrauterine environment differs drastically from wound healing in a clinical setting, where commensal dermatopathogens have direct access to sites of cutaneous injury. Thus, the early infiltration of neutrophils is critical to aid in eradication of the bacteria and debridement of the wound site. While this may delay repair and promote fibrosis, it is undeniable that these antimicrobial functions are crucial to control infection, and thus careful consideration should be taken with regards to the modulation of neutrophils.

Contrary to the work above, several other studies have demonstrated a need for neutrophil recruitment to the site of injury. In support of neutrophil-dependent wound healing, mice lacking the CXCR2 receptor responsible for neutrophil chemotaxis have an expected impairment in neutrophil recruitment to the site of injury [22]. Interestingly, this was accompanied by decreased re-epithelialization and angiogenesis [22]. These effects may be a direct reflection of the loss of CXCR2 on keratinocytes and fibroblasts, as this receptor is also important in their migration, in addition to neutrophil chemotaxis. It would be of interest to examine a conditional, tissue-specific knockout to further elucidate the respective contributions of CXCR2-mediated neutrophil recruitment and the migration of cells critical to re-epithelialization.

Aging studies have demonstrated delayed wound closure with increasing age, and also support the necessity for neutrophil infiltration for efficient wound closure as neutrophil depletion further delays wound closure in older mice [23]. In addition, topical application of isolated peritoneal neutrophils following injury or intravenous injection of granulocyte-colony stimulating factor (G-CSF) prior to wounding enhanced wound closure in aged mice to a comparable time frame seen in young mice [23]. These data provide an interesting point of contention in the argument regarding neutrophil necessity. The differential neutrophil requirement observed in fetal, young and aged mice may highlight an aging difference reflective of the immunosenescent phenotype of the elderly or the enhanced proliferative capacity *in utero*. Thus, in neutropenic or immunocompromised individuals incapable of recruiting adequate numbers of neutrophils to sites of injury, topical or systemic G-CSF may mitigate infectious complications. On the other hand, in young, healthy individuals,

decreasing the neutrophil flux at sites of injury by increasing macrophage infiltration and efferocytosis with use of mesenchymal stem cells [24] may improve rates of wound closure. While the absolute necessity of neutrophils in cutaneous injury remains in question, taken together, these data suggest that a delicate balance of neutrophil infiltration is required for efficient wound closure.

NKT cells as emerging modulators of cutaneous healing

Innate lymphocytes share properties of both the innate and adaptive immune system. In contrast to traditional lymphocytes, they possess an invariate germline encoded antigen receptor [25,26]. Often, this receptor displays some degree of autoreactivity to self-antigens, as well as foreign or microbial antigens [25]. For these reasons, innate lymphocytes play crucial roles in autoimmunity, infection and cancer immunology [26–29]. In addition, cutaneous wound healing reveals non-protein antigens that activate innate lymphocytes [28], either facilitating or impairing wound closure. Recently, one of these innate lymphocytes, the NKT cell, has been shown to participate in cutaneous wound repair, and their presence slows the pace of wound closure [28].

Natural killer T cells express an invariant TCR that recognizes glycolipid antigens presented in the context of CD1d molecules [30]. Although not resident cells of the skin, NKT cells infiltrate cutaneous wounds during the early inflammatory phase with kinetics similar to that of the neutrophil [31]. Similar to the laboratory studies with neutrophil depletion, the absence of NKT cells accelerates early wound closure. NKT cells regulate the local production of certain neutrophil and macrophage che-mokines, and in the absence of NKT cells, these mediators are transiently upregulated [31]. This highlights the alternative functions of chemokines as pro-fibrogenic, pro-angiogenic and pro-epithelialization agents. In particular, the presence of NKT cells at the site of cutaneous injury may directly enhance wound repair by attenuating the overexuberant neutrophil response. The NKT cell also regulates local TGF-β1 production. In their absence, early wound collagen deposition is increased [31]. Hence, NKT cells negatively regulate both inflammatory and fibroproliferative signals in the early wound.

Since the absence of NKT cells accelerates wound closure, they are attractive targets for novel wound-healing therapies. Systemic administration of a monoclonal antibody (anti-CD1d) resulted in a similar acceleration in early wound closure, as seen in NKT cell-deficient animals [32]. The most logical translation of this finding for clinical use would be topical delivery of anti-CD1d to problematic wounds, accelerating their closure. Aside from completely blocking NKT cell activation, there are numerous ligands now available to downregulate NKT cell activity [33–37]. Perhaps topical delivery of such ligands could accelerate wound closure without systemic toxicity or losing any potential antimicrobial function. Not only will our ability to modulate innate lymphocyte activity lead to novel wound healing therapies, but also our understanding of how these innate lymphocytes regulate the innate and adaptive immune responses at the wound site can provide more detailed, nuanced concepts of all the soluble mediators found within a healing wound.

Conclusion

The physiologic response to cutaneous injury is a complex, highly regulated process. Likewise, the contribution of innate immune cells to this process is multifaceted. While intact macrophage, DETC and mast cell function [6–15] have clearly been demonstrated to improve outcomes following cutaneous injury, neutrophils and NKT cells appear to play a much more deleterious role. However, none of the innate leukocytes can be seen as purely beneficial or harmful to the wound-healing process. As we have discussed, neutrophils have

important antimicrobial properties, whereas antimicrobial functions for the NKT cell at the wound site have yet to be discovered. Hence, the neutrophil might propagate a robust inflammatory response whose mediators might delay wound closure, while the NKT cell might regulate the magnitude of this neutrophil response. Furthermore, the soluble mediators associated with both cell types (such as the CXC chemokines) cannot be considered strictly proinflammatory. These mediators, and thus their impact on immune cell activation, have evolved to serve numerous angiogenic and fibrogenic functions, which under the proper circumstances, actually create wounds with increased breaking strength despite a slower closure time.

Since both cell types are among the earliest responders after injury, they serve as ideal targets for modulation of the wound repair process. Early intervention in high-risk patient populations using novel therapeutic strategies derived from our understanding of innate mediators of tissue injury can decrease the incidence and prevalence of chronic, nonhealing wounds, reduce infectious complications and ameliorate associated healthcare costs.

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