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Inflammation and wound healing

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1. ABSTRACT

Cutaneous wound healing is a complex process involving several overlapping phases. While we have made great strides in understanding these various phases, there is still much to learn about the cells and soluble mediators that are involved in a successful wound healing event. The current review describes the immuno/inflammatory cells and some less commonly studied soluble mediators involved in the adult healing response.

2. INTRODUCTION

Adult wound healing is a complex process involving cellular and biochemical processes including hemostasis, inflammation, proliferation and remodeling. This process starts soon after the wound occurs and can take several months to be completed. The result of this healing process in adult humans and higher vertebrate animals is the formation of a scar. However, during the first and second trimesters of gestation, both human and animal fetuses heal skin wounds without scarring (1). One key difference between the healing of fetal and adult skin is the level of inflammation after wounding. Unlike adult cutaneous wounds, fetal wounds heal in a scarless manner with a reduced inflammatory response (2). When a more intense inflammatory response is induced in fetal skin by the application of inflammatory mediators such as bacteria, reactive oxygen species or cytokines, the result is the formation of a scar similar to that seen in adult skin (3-6). These studies suggest that the recruitment of inflammatory cells to the wound site and the production of inflammatory mediators in the wound may play an important role in the type of healing that occurs. It is now believed that the adult inflammatory response may be a little overzealous in its attempt to prevent infection. While it achieves the goal of preventing infection this response also results in the suppression of regenerative healing, resulting in the formation of a scar (7). Besides age, other factors such as the site of the wound, as well as gender, also contribute to the type of healing that is seen. For example, wounds that occur in the oral cavity heal much faster than cutaneous wounds with a reduced infiltration of inflammatory cells, decreased cytokine production including TGF-beta and decreased scarring (8). It has also been shown that men

have an altered cutaneous inflammatory response, and as a result heal wounds more slowly than females (9). Since inflammatory responses facilitate the recognition and destruction of potentially dangerous pathogens and foreign materials, a delicate balance between decreasing the magnitude of the inflammatory response to reduce scarring and preventing infection must be maintained.

3. IMMUNO-INFLAMMATORY CELLS

Neutrophils, macrophages, mast cells and lymphocytes are the major cells involved in an inflammatory response following a wounding event. These cells play key roles in initiating and halting inflammation in the wound site, and mediate subsequent steps of the healing process (10).

3.1. Neutrophils

Neutrophils are the first inflammatory cell to arrive at the site of a cutaneous wound in response to multiple chemotactic signals including IL-1, TNF-alpha and IL-8 (11, 12). Bacterial products, such as lipopolysaccharides that can accumulate in wounds infected with bacteria can accelerate neutrophil migration into the wound site. The peak period of neutrophil infiltration into the wound site occurs between 24 and 48 hours following wounding (13). In the wound, neutrophils remove debris, foreign particles and bacteria (10, 13, 14) through phagocytosis, the production of oxygen radicals and the activation of complement (15). In addition, during their tenure in the wound site, neutrophils release several pro-inflammatory cytokines such as IL-1-alpha, IL-6, TNF-alpha, TGF-beta (16, 17) as well as PGE_2 (18). During the later phases of the inflammatory response, via a mechanism that is not well understood, neutrophils receive a signal to stop clearing the wound area, undergo apoptosis and are phagosytized by macrophages (19). Recent studies using murine antibodies to selectively deplete neutrophils confirmed older studies demonstrating that as long as sterile conditions were maintained, the loss of neutrophils did not negatively impact wound healing (20, 21). In fact, depletion of neutrophils accelerated the healing response. Studies from our laboratory demonstrated that daily topical application of the anti-inflammatory drug celecoxib to both incisional wounds (22) and excisional wounds (unpublished data) had no negative impact on re-epithelialization. The treatments did however decrease neutrophil infiltration into the wound site during the inflammatory phase, resulting in a significant decrease in scarring.

3.2. Macrophages

Macrophages infiltrate into the wound following neutrophils. These cells exhibit immunological functions as antigen-presenting cells and remove debris, foreign particles, bacteria as well as neutrophils that have undergone apoptosis (23-25). Peak macrophage presence in the wound is between 48-72 hours but they can remain present in the wound site for days to weeks. Macrophages play a key role in the transition between the inflammatory and proliferative phases through the release of growth factors and cytokines that mediate wound fibroplasia and neovascularization. As seen with neutrophils, macrophages produce pro-inflammatory cytokines such as IL-1- alpha, IL-1-beta, IL-6 and TNF-alpha (17). This cell type is also a source of PGE_2 as well as TGF-beta, FGF-2 and IGF (17, 26), which stimulate fibroblasts to produce collagen. New blood vessel growth results from macrophage release of VEGF-A, FGF-2 and TGF-beta (27). However, as with neutrophils there is evidence that macrophages may not be essential for wound repair. Studies carried out in PU.1-knockout mice, which lack both neutrophils and macrophages, show that these mice heal in the absence of fibrosis and without an inflammatory response, resulting in scarless healing similar to that seen in the fetus (28). These studies suggest that while macrophages may play an important role in the regulation of fibrosis and scarring, they may not be absolutely necessary for successful healing of a wound.

3.3. Mast cells

Mast cells are widely distributed throughout the body. They are found high numbers in organs such as the skin, lung and gut, which have contact with the external environment (29, 30). These cells localize near blood and lymph vessels as well as nerve fibers. Mast cells respond to activation from a variety of stimuli by releasing a wide range of biologically active mediators, both newly synthesized as well as those stored within their granules (31-33). While initially mast cells were identified as only participating in allergic responses, it is now clear that they participate in other conditions such as tumorigenesis, wound healing, and fibrosis (34-37). Unlike other cell types, it appears that the mast cell may play a key role in all phases of wound healing. In the early wound healing phases, mast cells enhance macrophage phagocytosis (38, 39) and appear to modulate neutrophil infiltration into the wound site (40). Mast cells also play a key role in coordinating neovascularization in the wound through the production and release of a number of factors including TNF, platelet derived growth factor (PDGF) vascular endothelial growth factor (VEGF) and fibroblast growth factor (FGF) (41, 42). Mast cells also have a profound effect on fibroblasts. Factors produced by mast cells activate fibroblasts, promoting collagen synthesis thus aiding in the deposition of a temporary matrix within the wound. Mast cells also participate in the formation of a permanent matrix during the remodeling phase through a balanced release of both degrading enzymes and growth factors (43-45).

3.4. T-cells

The exact role of the T-cell in the healing response is not yet clear. These cells appear in the wound site after wound closure has occurred and any infections have been cleared. T-cells appear to mainly modulate the healing response through the production of cytokines and growth factors (46). T-cells have been associated with tissue fibrosis and excessive hypertrophic scar formation (47, 48). Early studies using the athymic nude-*nu* mouse, which is T-cell deficient, found that this mouse healed with less scarring than the wild type controls (49). A more recent study compared cutaneous wound healing in athymic nude-*nu* mice, wild-type C57BL/6J controls, thymectomized C57BL/6J mice and C57BL/6J mice treated

with the immunosuppressant cyclosporine A (50). These experiments showed that only the nude-*nu* mice were able to heal without forming a scar. The major differences between these mice and the other immunodeficient animals were a lack of $CD8^+$ T-cells in circulating blood and wounded skin and low levels of pro-scarring cytokine TGF-beta1 and PDGF-B. While the loss of $CD8^+$ T-cells appears to benefit healing, the loss of $CD4^+$ T-cells decreases wound tensile strength (51). These studies suggest that the healing response can be modulated depending on the presence or absence of specific subsets of T-cells. Recently Jameson et al have documented a role for gamma delta dendritic epidermal T cells (DETC) in wound repair (52, 53). These authors reported that mice lacking DETCs had an impaired epidermal cell proliferation and a significantly delayed wound healing response. It is possible that these cells participate in the repair of wounds through the production of factors including FGF-7 and FGF-10.

4. GROWTH FACTORS AND HORMONES

It is well accepted that growth factors play a significant role in the successful wound healing process (16). Some of the most studied growth factors, including platelet-derived growth factor (PDGF), transforming growth factor-beta (TGF-beta), and fibroblast growth factor (FGF) (54) are known to be involved in wound healing. However, the contribution of other growth factors as well as the role of hormones is less well understood.

4.1. Cyclooxygenase-2 and Prostaglandin E_2

One of the early responses to an inflammatory stimulus such as wounding is the induction of the cyclooxygenase-2 (COX-2) enzyme, which catalyzes the conversion of arachidonic acid to prostaglandins (55). Inflammatory stimuli induce the release of arachidonic acid from membrane phospholipids by inducing the activity of phospholipases and also induce the production of the COX-2 enzyme, both of which contribute to an increase in prostaglandin E_2 (PGE_2) and other arachidonic acid pathway end-products during inflammatory events. Previous studies have described the induction of COX-2 in healing wounds (56, 57). The COX enzymes, which include the homeostatic COX-1 enzyme in addition to the inflammatory COX-2, are the targets of nonsteroidal anti-inflammatory drugs (NSAIDs) such as aspirin and ibuprofen. Based on the side-effects associated with the use of these non-selective NSAIDs, which have been attributed to COX-1 inhibition, specific COX-2 inhibitors such as celecoxib (Celebrex®, Searle, St. Louis, MO) were developed. These inhibitors specifically target the COX-2 enzyme, preventing its enzymatic activity and the subsequent production of prostaglandins (58).

Although the role of prostaglandins in the scar formation phase of healing has not been well described, several studies have demonstrated the ability of prostaglandins to induce fibroblast proliferation *in vitro* and collagen production in wounds *in vivo* (59-61), suggesting a pro-fibrotic role for PGE_2 in the skin. However, the contribution of PGE_2 to fibrosis may be tissue specific, since studies suggest that PGE_2 can inhibit the production of type I collagen alpha1 by human embryo lung fibroblasts (62). This information, in conjunction with what is known about scarless fetal healing, suggests that prostaglandins and the inflammatory response induced by these mediators, at least in part, control the amount of fibrosis that will occur following wound repair in adult skin. We have shown that celecoxib applied directly to adult wounds inhibits several parameters of inflammation. Decreasing the magnitude of the early inflammatory phase of wound healing had a significant effect on later events in the wound healing process, resulting in decreased scarring without disrupting re-epithelialization or reducing tensile strength (22). Additional studies from our laboratory using a murine fetal wound healing model demonstrated differential expression of the COX-2 enzyme in early and late gestation fetal wounds and showed that the introduction of PGE_2 into early fetal wounds caused a delay in healing and the production of a scar (6). These studies further support a role for the COX-2 pathway in scar formation.

Others have examined the efficacy of oral dosing with "NO-NSAIDs" i.e. an NSAID coupled with a nitric oxide-releasing moiety, on the healing response. These studies were based upon previous reports of the beneficial effect on healing of administration of nitric oxide donors or transfection of a wound with the gene for inducible nitric oxide synthase (63, 64). The NO-NSAID, HCT-3012, significantly enhanced collagen deposition at the wound site, however the effects of this compound on scar formation were not described (65).

4.2. Connective Tissue Growth Factor

Connective Tissue Growth Factor (CTGF) is present during wound healing in a number of tissues, including the skin (66, 67). In the skin, it is produced by fibroblasts following stimulation by TGF-beta (66) and appears to act as an autocrine growth factor. Biological effects of CTGF include stimulation of proliferation, angiogenesis, migration, extra-cellular matrix (ECM) production, cell attachment, cell survival and in some cell types apoptosis (68, 69). In adult mammals, this growth factor is expressed at high levels during wound repair and at sites of connective tissue formation (70). Takehara et al suggested that in fibrotic skin, while TGF-beta induces fibrosis, CTGF acts to maintain tissue fibrosis. CTGF appears to be indirectly regulated by PGE_2. Recently, Ricupero et al demonstrated that PGE_2 inhibits the TGF-beta stimulated increase in CTGF transcription (71). Thus manipulating PGE_2 levels could result in a decrease in CTGF production and ultimately in a decreased fibrotic response. However, further studies are necessary to determine the exact nature of this interaction. While CTGF appears to be a good target candidate for anti-fibrotic therapy, the exact contribution of this growth factor to the normal healing response or its role in chronic wounds is currently unclear.

4.3. Estrogen

Estrogens are C-18 steroids synthesized from cholesterol in the ovary prior to menopause and in the peripheral tissue such as the skin in postmenopausal

women (72). Two estrogen receptors, alpha and beta, have been cloned and found to be expressed by a variety of cells including fibroblasts, macrophages, endothelial and epidermal cells of the skin (73). It has been shown that estrogen can affect these same cells (reviewed in (74)). While the effects of estrogen on aging of the skin have been studied for several years, only recently has the importance of this hormone in the healing response been evaluated. Based upon a number of studies, estrogen appears to affect *in vivo* cutaneous wound healing by modulating inflammation. This includes a dampening of the inflammatory response by the inhibition of neutrophil infiltration and a decrease in the production of pro-inflammatory cytokines, as well as a decrease in macrophage inhibitory factor (MIF) and enhanced deposition of collagen (74-76). *In vitro* studies demonstrated that exposure of keratinocytes to estrogen increased proliferation of these cells and increased GM-CSF production, resulting in enhanced re-epithelialization (77, 78). Using a rodent model, Ashcroft et al (79) demonstrated that the application of estrogen topically accelerates cutaneous wound healing of incisional wounds, most likely via the stimulation of increased levels of TGF-beta1 in the wounds. The mechanism by which estrogen leads to increased TGF-beta1 levels is not yet completely understood. Additional studies by the same group demonstrated that the topical administration of estrogen to wounds of elderly men and women resulted in reduced local inflammation, decreased wound sizes, increased collagen and fibronectin levels, and enhanced wound strength (80). Thus it appears that a threshold level of this hormone is necessary within the skin not only to regulate cutaneous aging but also for successful healing.

4.4. Androgens

Androgens have been reported to be critical mediators of immune responses in a number of diseases (81, 82). Based upon these studies it appears that depending on the cell type and model, androgens may have both pro- and anti-inflammatory effects. While it is clear that at least in the elderly, estrogen accelerates repair in both human and animal models, the importance of androgens in the healing process is less well understood. As with estrogen, the skin has been classified an androgen sensitive organ. The skin expresses the two genes encoding 5 alpha-reductase, which converts testosterone to its more active metabolite, 5 alpha-dihydrotestosterone (83). Both enzymes signal through the androgen receptor, which is believed to be expressed by resident skin cells including epithelial cells and fibroblasts. Ashcroft et al demonstrated that castration of mice resulted in a dampened inflammatory response, increased matrix deposition and accelerated cutaneous wound healing (84). Furthermore, similar to the effects of castration, treatment with an androgen receptor antagonist decreased the inflammatory response within the wound and resulted in accelerated healing. Further studies from the same group have shown that the administration of androstenedione to castrated mice exaggerates the inflammatory response, reducing the rate of wound healing (85). These studies suggest that modulating the production of testosterone may prove to be a potential therapeutic target for inducing successful wound healing.

5. PERSPECTIVE

In summary, while it is clear that many cell types, growth factors and hormones play important roles in successful healing, we still have a way to go to truly understand how get a wound to heal quickly and with minimal scarring. It appears that in healthy individuals in the absence of infection, an overly robust inflammatory response occurs after wounding. This vigorous inflammatory response may no longer be necessary with the development of antibiotics and higher sanitary standards. In addition, this overactive inflammatory response appears to augment scar formation and can also extend the time it takes to heal a wound. Continued studies designed to increase the understanding of how inflammatory cells as well as the soluble factors involved in the inflammatory response ultimately affect healing and scar formation are needed. This information could lead to the development of better treatment modalities for modulating scarring as well as for the treatment of chronic wounds.

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