Wound Repair

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WOUND REPAIR

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Following injury, a series of events is initiated that includes global and local reactions. Global reactions, such as inflammatory and immunological responses as well as adjustments in neural and endocrine status, are directed at marshaling the organism's resources for dealing with changes in its integrity and the potential threat of infection or other complications. Injury entails cell and tissue damage and often a physical breach in the barrier against the outside world (e.g., skin). Local reactions are exemplified by immediate hemostatic (e.g., blood clotting) events followed by changes in local cellular composition created by the inflammatory infiltrate and adjustments in resident cell function. These are accompanied by local metabolic adjustments. These events are directed at restoring local integrity and establishing a relevant steady-state.

The typical events of wound repair are extensively documented and well characterized. In recent years, research has explored regulation of wound repair at the cellular level and has sought alternative modes for correcting tissue damage that yield more efficient restoration of preinjury conditions (e.g., regeneration). Since repair typically leads to replacement of damaged tissues with connective tissue, reduction in function invariably accompanies wound healing. Where tissue damage is slight, this causes little or no problem for the individual. However, when tissue damage is great, as for example when a finger or limb is lost, the compromise of wound repair carries a noticeable price (both in actual costs and in quality of life for the affected individual).

HALLMARKS OF INFLAMMATION AND STAGES OF WOUND REPAIR

Interest in wound healing and its management has a long history.[1,2] While ancient physicians did not systematically study the basic biology of wounds; they, nevertheless, realized that wounds shared hallmark characteristics. In particular, the Egyptians and Greeks clearly recognized inflammation and its association with wounds.[3] Moreover, by the first century A.D. the four cardinal signs of inflammation had been described by Celsus.[3] These features, "... rubor et tumor cum calore et dolore ..." (redness and swelling with heat and pain), had become clearly linked to wounds. More importantly, the manner in which these signs appeared and their association with other symptoms both directed prescribed courses of treatment and predicted potential outcome. By the mid-nineteenth century, a fifth feature was recognized. This feature (functio laesa [disturbed function]) was added by Rudolf Virchow, the founder of modern cellular pathology. At about this time, studies by Julius Cohnheim led to a physiological explanation for these characteristics of inflammation. He observed that vasodilation could account for the rubor (redness), that increased blood flow could produce the calor (heat), and that exudation of fluid from local blood vessels gave rise to the tumor (swelling) of inflammation. In addition, he inferred that these factors collectively contributed to the dolor (pain) that accompanies wounds.

Wound repair can be divided into several overlapping phases. Some recognize four phases (hemostasis, inflammation, proliferation, and remodeling)[4] while others identify three (inflammation, reepithelialization and granulation tissue formation, matrix formation and remodeling).[5] These schemes differ more in what is emphasized than in substance. For the discussion below, we consider three intervals: intiation (hemostasis and inflammation), progression (proliferation and matrix production), and resolution (remodeling).

INITIATION OF REPAIR — HEMOSTATIC AND INFLAMMATORY PHASES

Hemostasis is initiated immediately after injury. It involves constriction of injured blood vessels and formation of a fibrin clot. These events are directed at limiting blood loss. In addition, they trigger early neural, endocrine, and hematological responses. Hemostatic events reach their peak early and subside within a few days. Hematological events that are activated by hemostasis[6] give rise to inflammatory and immunological responses that characterize the inflammatory phase of repair. This phase is characterized by infiltration of various leukocytes into the wound site. Inflammation follows a similar course in all acute inflammatory states and consists of three stages. The first stage is characterized by an initial neutrophil (polymorphonuclear leukocytes, PMNs)-rich infiltrate that is soon replaced by a monocyte/macrophage (Mϕ)-rich infiltrate. PMNs enter the wound within hours of injury and dominate during the first one to two days. (Persistence of PMNs in large numbers beyond this time suggests complications, such as infection, and often leads to delayed or impaired healing.) Mϕs begin entering the wound one to two days after injury. The period of Mϕ predominance marks the second stage of the inflammatory phase. It coincides with the initiation and expansion of the proliferative phase of wound repair. The third stage of the inflammatory phase is characterized by infiltration of lymphocytes into the injury site. The inflammatory phase persists as long as leukocytes continue to be recruited to the site of injury. For example, in simple dermal wounds, this phase might conclude within one week of injury, by which time the numbers of leukocytes in the wound begin to decline (see figure 1 in reference 4).

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Initiating wound repair in adult mammals

Provisional matrix

Immediately following injury, a provisional matrix is created.[5] This matrix is initially provided by the fibrin clot but is subsequently replaced by a matrix produced by local cells. Provisional matrix contains fibrin (the initial clot) as well as fibronectin, vitronectin, thrombospondin, and other substances that promote cell adhesion and migration, serve as a reservoir for other bioactive substances (e.g., growth factors, proteases, and protease inhibitors), and modulate responsiveness of local cells to these latter agents.[5,6] For example, collagen attenuates transforming growth factor beta (TGF-β) stimulation of collagen production by fibroblasts while fibrin permits TGF-β induced collagen production and enhances platelet-derived growth factor (PDGF)-promoted expression of integrins $\alpha_3\beta_1$ and $\alpha_5\beta_1$ which are associated with granulation tissue. This suggests that provisional matrix supports, if not promotes, display of a granulation tissue phenotype by fibroblasts at the site of injury.

Growth factors and cytokines

Metchnikoff[7] recognized the importance of phagocytic Mφs. Recent studies have demonstrated their role as an important source of growth factors and cytokines[8,9] many of which are involved in wound repair. Growth factors active in repair include: epidermal growth factor (EGF) and TGF-α,[10] the TGF-βs,[11] members of the fibroblast growth factor (FGF) family,[12] PDGFs,[13] and insulin-like growth factor (IGF) I.[14] In addition, numerous cytokines and chemokines also promote repair.[14-18] These include cytokines like tumor necrosis factor (TNF)-α, interferon (IFN)-γ, interleukin (IL)-1, IL-6, IL-8, and IL-10 and chemokines such as monocyte chemoattractant protein (MCP)-1, and Mφ inflammatory protein (MIP)-1. Moreover, growth hormone[19] and angiotensin II[20] also promote repair. This is a small sampling of agents found at sites of injury that promote wound repair.

These factors play overlapping and reinforcing roles in promoting repair. For example, the proinflammatory cytokines IL-1, MCP-1, the MIPs, and TNF-α attract leukocytes to the wound site and modulate inflammatory cell responses after they arrive. TGF-β also can act as a chemoattractant drawing inflammatory cells to the site of injury. In addition, angiogenesis is promoted by several cytokines (e.g., IL-1, IL-8, and TNF-α) and growth factors (e.g., FGFs, PDGFs, and TGF-βs). Acting as proinflammatory and angiogenic factors, these agents ensure steady traffic of inflammatory cells, nutrients, and other resources to the site of injury.

Fetal wound healing is associated with a weaker inflammatory reaction

In adult mammals, injury provokes reactions that lead to replacement of normal tissue with a connective tissue scar. However, scarless repair can occur in fetal mammals. This seeming restoration, rather than replacement, of damaged tissue has raised hope that understanding differences between fetal and adult repair processes might provide insights into how regenerative potential might be expressed (or awakened) in adults. [See Stocum's[21] contribution in this issue for other discussion of this topic.]

In the fetus, following injury, the characterisic inflammatory picture described above does not occur. Fetal wounds are notable especially for the conspicuous absence of PMNs.[22-24] The acute inflammatory reaction appears to be considerably diminished, if not absent. Nevertheless, mononuclear inflammatory cells (i.e., Mφs and lymphocytes) characteristic of the later inflammatory reaction appear during fetal wound healing, although the number of Mφs is diminished. It is unclear why PMNs are absent from and Mφs diminished in fetal wounds. Nevertheless, one cannot ascribe this to an inability of PMNs or Mφs to be attracted to a site of injury because of relative immaturity. PMNs and Mφs will infiltrate sites of injury if suitable provocation exists, for example, in the form of chemoattractants[22] or massive cell destruction.[25]

Fibrin clots are typically absent from fetal wounds and platelet degranulation occurs poorly.[23] Platelet degranulation delivers several factors, including EGF, PDGF, and TGF-β to the wound. In addition, the fibrin clot can retain growth factors at the site of injury. Thus, poor platelet degranulation and the absence of a fibrin clot could affect growth factor availability. This, in turn, can affect Mφ activation and stimulation of fibroblasts to proliferate or produce collagen. In this regard, Whitby and Ferguson[26] observed early disappearance of PDGF and the absence of TGF-βs and basic FGF from fetal mouse wounds when compared to wounds in neonatal or adult mice. Since these are fibrogenic growth factors, a correlation between their absence and limited scarring is not surprising.

The suggestion that an altered inflammatory response during fetal wound healing is a major contributor to the regeneration-like response observed is intriguing. This parallels the suggestion that inflammatory and immunological responses of amphibians during regeneration of appendages differ from those observed in animals undergoing nonregenerative wound repair.[27,28] However, little yet is known of which, if any, of these differences are material to the postinjury outcome.

PROGRESSION — PROLIFERATIVE AND MATRIX PRODUCTION PHASE

Progression towards repair is achieved through cell proliferation and accumulation of extracellular matrix (ECM). As this occurs, new tissue is produced to replace the damaged tissue. This period is characterized by active angiogenesis and neovascularization (formation of new blood vessels), fibroplasia (proliferation of fibroblasts), and ECM production. These events are driven by cytokines and growth factors from several sources, e.g., cells of the inflammatory infiltrate, primarily Mφs, vascular endothelium, and even fibroblasts themselves. The consequence of these events is replacement of damaged parenchymal cell mass by connective tissue. The altered ratio of supportive connective tissue to parenchymal cells gives rise to a new steady-state. This state might have little effect on function; however, when the amount of connective tissue becomes substantial, function is compromised. For example, replacement of damaged skeletal muscle by connective tissue will impair the contractility and contractile strength (force) of a muscle.

Participation of the inflammatory infiltrate

Metchnikoff[7] recognized the important role of Mφs as phagocytes. More recent studies have described a broader range of functions which include cytotoxic roles mediated by degradative enzymes and reactive oxygen intermediates (e.g., nitric oxide, NO) as well as growth promoting roles mediated by growth factors and cytokines[8,9] (noted above). Carrel[29,30] recognized the growth promoting role of other leukocytes in repair processes. This notion was echoed for regeneration by

Liebman[31,32] and Prehn.[33] More recently, it has been suggested that mast cells might play a more central role in wound repair than had been originally believed.[34] In contrast to Metchnikoff, these latter authors stressed contributions by cells with lymphocyte or granulocyte features. Nevertheless, all emphasized contributions to generation of new tissue by soluble cell products capable of modulating proliferation or matrix deposition at the site of injury.

Progress into the proliferative phase of repair does not depend on PMNs[35,36] but is affected by blocking or delaying Mϕ infiltration into the injury site.[36,37] In addition, the quality of repair, as determined by increased collagen content or tensile breaking strength, correlates well with Mϕ infiltration or activation.[38-41] Lymphocytes, especially thymic lymphocytes (T-cells), also affect the quality of repair.[42-44] In particular, cytotoxic/suppressor T-cells (CD8^{+}) are believed to play a counterregulatory role in repair. This is supported by observations that depletion of all T-cells reduced wound breaking strength and hydroxyproline content (a measure of collagen abundance) of wounds.[44] Moreover, selective depletion of CD8^{+} T-cells enhanced healing while selective depletion of CD4^{+} T-cells (helper T-cells) did not affect wound healing outcome.[43]

Contribution of growth factors and cytokines

As noted above, some cytokines and growth factors are proinflammatory and angiogenic. In contrast, others are fibrogenic since they promote connective tissue accumulation.[16] These include FGFs, IGF-I, IL-1, PDGFs, TGF-βs, and TNF-α which stimulate fibroblast proliferation in vitro as well as collagen synthesis or actual fibrosis in vivo. Their actions are antagonized by other growth factors or cytokines. For example, IL-6 and MCP-1 can reduce fibroblast proliferation, IL-8 diminishes collagen synthesis, and IFNs can reduce fibroblast proliferation in vitro and both collagen synthesis (IFN-β and IFN-γ) and fibrosis in vivo (IFN-γ).

Altered availability of important cytokines and growth factors would be expected to affect the progress of wound repair. Indeed, recently Fivenson and associates[18] monitored up to 16 chemokines and inflammatory cytokines in a group of individuals undergoing therapy of chronic leg ulcers. As wound healing progressed, angiogenic factors (e.g., neutrophil-activating peptide-2 and IL-8) tended to become prevalent over angiostatic factors (e.g., IFN-γ-inducible protein 10 and platelet activating factor 4). Moreover, in an independent comparison of growth factor and cytokine content in mastectomy fluids (representing an acute resolving wound) with fluids from chronic wounds (nonhealing), Tarnuzzer and Schultz[45] found that IL-1β, IGF-I, TGF-α, TGF-β, and TNF-α were generally higher in fluids from chronic wounds. In contrast, they found that EGF content was lower in these fluids. These observations would be rather perplexing because of the fibrogenic nature of these growth factors were it not for the accompanying observation that fluids from chronic wounds also contained substantial protease activity.[45] Thus, chronic wound fluids appear to have enhanced capacity for collagen degradation and the potential for degrading or inactivating growth factors.

Manipulating growth factors (e.g., EGF, FGF, IGF-I, PDGF, and TGF-βs) has had beneficial effects on wound repair in diverse models. However, in many cases treatment with growth factors has yielded disappointing results. Success (or failure) of growth factor therapy in these cases likely is attributable to the fit between the solution and the problem. Robson[46] stresses that addition of a growth factor which is not already absent or deficient or presentation of an agent that does not stimulate appropriate responses cannot improve the quality of repair.

Influence of fibroblast type

Wound repair is profoundly dependent upon fibroblasts, as noted above. However, several studies have shown that fibroblasts are not homogeneous.[47-49] Fibroblasts differ especially in morphology, response to bioactive substances, growth potential, and collagen production. Some of these differences are age-related[50] while others are probably environmental[51] or the result of developmentally distinct histories. Such differences can affect the quantity or quality of repair.

Fetal fibroblasts, unlike adult fibroblasts, migrate into three-dimensional collagen gels.[52] This is promoted by a migration stimulating factor produced by fetal fibroblasts. This factor, which is also produced by tumor fibroblasts but not normal adult fibroblasts, promotes fibroblast migration and stimulates hyaluronan production.[53,54] The maintenance of cell mobility and high hyaluronan levels are believed to be factors contributing to the absence of scarring in fetal wounds.

More recently, Mast, et al.[55] have noted that fibroblasts in fetal rabbit skin appear active, containing numerous secretory vesicles, unlike adult skin fibroblasts which display an essentially quiescent ultrastructural morphology. Consequently, there is little discernible change in the ultrastructural features of fetal fibroblasts as they are engaged for wound repair. This contrasts markedly with the apparent change in adult fibroblasts to an activated state following injury. It, thus, appears that fetal fibroblasts are in a different dynamic state than adult fibroblasts prior to injury. The occurrence of fetal fibroblasts in an already activated state suggests that the regeneration-like repair of fetal wounds represents an expansion of activities already in progress rather than an attempt to recapitulate events formerly inactivated (as in adult wound repair).

Gross[56] has suggested that the type of fibroblast recruited into adult wounds affects the quality of repair. In particular, hypodermal fibroblasts normally participate in healing wounds to the skin whereas dermal fibroblasts remain inactive. Properties of these cells differ markedly. Gross speculates that a portion of the dermal fibroblast population may retain stem cell properties. In addition, he suggests that the inability to release dermal fibroblasts from their matrices, as occurs in regenerating amphibian limbs, might be an impediment to regeneration-like response to injury in adult mammals.

RESOLUTION

As wound repair resolves, relative quiescence must be restored to the tissue and a steady state comparable to that of the surrounding tissue must be reestablished. The remodeling phase of repair represents this period of final resolution. This interval can last for several weeks to months. During this time, inflammatory cells no longer enter the wound site but rather are diminishing in abundance. Consequently, the events which they promoted subside. In addition, cell proliferation (primarily of fibroblasts) is on the decline. Indeed, during this time programmed death (apoptosis) also contributes to reducing the cellularity of the wound site.[57] Furthermore, progressive accumulation of ECM is replaced in favor of its remodeling (restructuring); hence the name for this phase. The content of matrix components like hyaluronan and fibronectin decreases

while collagen type I accumulates and altered collagen crosslinking increases tensile strength of the scar during this time.[57] The dynamic state that had formerly typified this area is replaced by one of relative calm. Cellular activities are no longer directed towards tissue generation and renewal but rather are focused on maintenance. Except for an altered ratio of connective tissue to parencymal mass, normal structure and function are regained.

SYNOPSIS

The foregoing discussion examined several aspects of mammalian wound repair. Emphasis was placed on inflammatory cells, polypeptide growth factors and cytokines. In order to limit the scope of our discussion, numerous topics were omitted. These include, for example, the role of the extracellular matrix and of metabolites within the wound environment on the several events of wound repair. All of these components are instrumental in promoting the initiation and progress of wound repair. Ongoing studies are extending further our understanding of the nature and contributions of bioactive factors and of cell interactions to regulation of cell proliferation and ECM production and remodeling. Many of these studies are directed at improving the quality of wound repair, particularly in instances where healing is impaired. Other studies are focused on redirecting the pattern of events to achieve a different resolution, namely regeneration.

STEM CELLS — A TOOL FOR REPAIR, A PROMISE FOR BETTER OUTCOMES

Responses to injury, whether leading to repair or regeneration, rely on effector cells. One difference between repair and regeneration as resolutions to injury might be the consequence of selecting between two alternative classes of potential effector cells.[58] One of these, the fibroblast, produces connective tissue while the other, a parenchymal cell or its precursor, enables regeneration to occur. The notion of a precursor cell from which specific tissue types arise is not a novel concept, it has been around for decades. These precursors are called stem cells. Stem cells are defined as lineage-restricted or pluripotent depending upon whether they give rise to a specific adult cell type (lineage-restricted) or several cell types (pluripotent). Skeletal muscle satellite cells are an example of a lineage-restricted stem cell since they normally differentiate only into skeletal myocytes (and fuse to form skeletal muscle fibers). On the other hand, hemopoietic stem cells are pluripotent since they are capable of differentiating into any of the adult blood cell types.

The ability to isolate, manipulate, and exploit stems cells would help improve the quality of tissue restitution after injury and in developing artificial organs. Contributions to and uses of stem cells in regeneration or tissue engineering are explored by other articles in this symposium.[21,59]

While it is not within the scope of this article to explore these questions in detail, it is appropriate, nevertheless, to consider certain related questions. For example, some believe that stem cells are widely distributed in postembryonic tissues.[60,61] Why then, is regeneration so rare? If cells that retain the potential for restoring parenchymal mass exist within tissues, why does regeneration not occur routinely? Although no answer is yet available, it is reasonable to suggest that regeneration, rather than repair, does not occur because either these cells cannot adopt an appropriate phenotype or that local events present impediments to this potential being realized.

Results of studies from diverse sources favor the second explanation. For example, putative stem cells from different connective tissues can be induced to express numerous phenotypes. Lucas, et al.[62] have reported that cells derived from rat muscle can display features of bone, cartilage, endothelial, fat, skeletal muscle, and smooth muscle cells in culture. Their study cannot establish whether one or more precursor cell types contributed to the phenotypes observed; nevertheless, their study suggests that tissues might have broad adaptive potential. In addition, their study raises questions of how the local environment and signals within it contribute to final expression by these cells. Recently, myoblasts (muscle stem cells), which participate in forming new muscle fibers during skeletal muscle regeneration,[63,64] were prevented from forming myotubes both in vivo and in vitro by a mammalian wound repair environment.[65,66] These latter observations suggest that the (normal) wound repair environment is aptly suited to ensure formation of a connective tissue scar but that it differs from an environment which supports regeneration.

Ongoing studies of stem cells raise the hope that an untapped potential exists for better resolution to injury in the future. Tapping this potential requires better understanding of the properties of stem cells, on the one hand, and a greater understanding of both the nature of the injury environment and of the manner in which this environment influences the behavior of resident cells, on the other.

ACKNOWLEDGEMENTS

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