The Commonality of Cutaneous Wound Repair and Lung Injury*

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Soluble mediators, formed blood elements, extracellular matrix (ECM) proteins, and parenchymal cells integrate in dynamic interactive processes to effect normal repair of injured tissue. Unencumbered, these processes follow a specific time sequence. These wound repair events can be temporally grouped into inflammation, tissue formation, and tissue remodeling; however, these phases of wound repair are not mutually exclusive but overlap in time.

In higher vertebrates, tissue loss that disrupts normal architecture precludes tissue regeneration. New tissue formation consists of a fibroproliferative response that is usually remodeled into a fibrotic scar. When injurious agents persist or recur, the initial inflammatory phase continues, causing further tissue damage and prolongation of repair. Such persistent or recurrent inflammation may lead to a nonhealing wound or excessive fibrosis.

Abnormal healing may also result from maladaptive normal repair processes. For example, diffuse alveolar or diffuse bronchiolar injury elicits an apparently normal fibroproliferative response. The result in the lung, however, is the acute respiratory distress syndrome or obliterator bronchiolitis, respectively. If the process culminates in lung fibrosis, lung function may be progressively impaired. Widespread skin injury such as extensive burns also can result in maladaptive scarring. Other fibrotic disorders, such as progressive systemic sclerosis (PSS), do not appear to involve the fibroproliferative response but rather a direct stimulus for fibrosis; nevertheless, extensive maladaptive scarring occurs in multiple organs, including the skin and the lungs.

These maladaptive syndromes of fibrosclerosis underscore the likelihood that wound repair was teleologically designed to provide a localized adaptive patch for traumatic organ injuries sustained from hostile environmental sources such as the saber tooth tiger or an unfriendly warrior. However, when widespread fibroproliferative or other sclerotic processes are elicited in the lung or skin by viral, chemical, physical, or immunologic injury, the extensive scarring severely impairs normal lung and skin function.

The influx of granulation tissue into a gaping traumatic wound and its maturation to scar probably involve the same processes that produce the fibroproliferative buds observed in alveoli or bronchioles after diffuse lung injury and the sclerotic changes observed in burns and PSS. The former is adaptive, the latter are maladaptive (Fig 1). In the context of a brief overview of cutaneous gaping wound repair, certain key regulatory events leading to the fibroproliferative response and the resulting fibrosis will be emphasized and related to maladaptive sclerosis. A more comprehensive review of cutaneous wound repair has been published recently.1

INFLAMMATION

Injury initiates inflammation by cell death and disruption

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**FIGURE 1.** Scheme of events in wound healing. The phases actually overlap in time. Tissue loss that disrupts normal architecture precludes tissue regeneration and repair is achieved by the fibroproliferative responses, which may also produce abnormal or maladaptive results.

of blood vessels resulting in extravasation of blood constituents, platelet activation, blood coagulation, and mediator generation. Platelets first adhere to interstitial connective tissue, then activate, aggregate, and induce blood coagulation. In addition, platelets release biologically active substances: ECM molecules such as fibronectin, fibrinogen, thrombospondin, and von Willebrand factor, which facilitate platelet aggregation and binding to connective tissue; growth factors such as platelet-derived growth factor (PDGF), transforming growth factor-α (TGF-α), and TGF-β, which promote new tissue generation; and vasoactive substances such as serotonin, adenosine diphosphate, calcium, and thromboxin, which stimulate blood vessel constriction and additional platelet aggregation, thus curtailing hemorrhage.

Proenzymes of the plasma coagulation system absorb onto interstitial connective tissue components and activated platelet membranes and self-activate. The activation of this system rapidly leads to clot formation. In the absence of thrombogenic surfaces, plasma coagulation proenzymes are literally afloat in a sea of enzyme inhibitors. Thus, although small amounts of these proenzymes are activated continuously under normal circumstances, the enzymes are immediately quenched by plasma protease inhibitors. However, when the proenzymes have been absorbed onto a surface in a microenvironment relatively free of protease inhibitors, minute amounts of spontaneous activation are quickly amplified into the physiologic response of blood clotting.

The balance between plasma enzyme inhibitors and the availability of thrombogenic surfaces is one mechanism by which blood coagulation is tightly regulated. In addition, several intrinsic activities of intact blood vessel endothelium limit the extent of platelet aggregation and blood coagulation to the wounded area. These include production of prostacyclin, which inhibits platelet aggregation; cell surface inactivation of thrombin; cell surface activation of protein C, in enzyme that degrades coagulation factors V and VIII; and generation of plasminogen activator, which initiates clot
A variety of biologically active mediators are generated from the coagulation cascade including kallikrein, thrombin, plasmin, fibrinopeptides, fibrin split products, bradykinin, and the anaphylatoxins C3a and C5a through spillover activation of the complement cascade. Besides vasoregulatory and clotting functions, most of these factors have leukocyte chemotactic activity and thereby promote recruitment of circulating leukocytes to the site of injury. Thrombin and plasmin also promote parenchymal cell growth.\textsuperscript{9}

Generation of leukocyte chemotactic factors is one mechanism to induce diapedesis of leukocytes to a site of injury. In addition, the endothelium at the site may be activated to express leukocyte adhesion receptors to facilitate leukocyte transmigration across the endothelial barrier. Endothelial cells activated by a variety of cytokines insert in their surface membranes specific molecules that blood leukocytes recognize, such as intercellular adhesion molecule (ICAM), endothelial-leukocyte adhesion molecule (ELAM), and granule membrane protein of 140 kd molecular weight (GMF-140).\textsuperscript{10,11} These endothelial cell molecules are recognized by specific receptors on the surface of leukocytes including the $\beta_2$ integrin family of receptors (MAC-1, LFA-1, and p150) (Table 1) as well as other molecules yet to be identified. Circulating leukocytes upregulate $\beta_2$ integrin receptors upon activation by chemotactic factors. Through this cell-cell interaction, leukocytes adhere to and migrate through blood vessels at sites of injury.

Within the first few hours neutrophils infiltrate the wound, followed by monocytes 24 to 48 h later. Although both cell types are attracted to wound sites by chemotactic factors generated through coagulation cascade activation, monocytes are also recruited by fragments of degraded ECM proteins.\textsuperscript{12}

Neutrophils function mainly to rid the tissue of contaminating bacteria. In doing so they often release lysosomal enzymes and other toxic substances that cause additional tissue destruction. Although excessive tissue destruction clearly delays tissue repair, dead and dying cells do release a variety of substances, such as tissue factor, lactic acid, lactate dehydrogenase, calcium, lysosomal enzymes, and fibroblast growth factor (FGF), that promote healing.\textsuperscript{13}

Monocytes invading the wound site quickly undergo a metamorphosis to macrophages, which phagocytose and kill pathogenic organisms and scavenge tissue debris, including effete neutrophils.\textsuperscript{14,15} They also release chemotactic factors that recruit additional inflammatory cells, and enzymes that augment tissue degradation. In addition, macrophages release a second wave of growth factors, including PDGF, TGF-\(\alpha\), and TGF-\(\beta\). As mentioned before, these factors are critical for new tissue formation.\textsuperscript{16,17} We have recently discovered that monocytes are stimulated to express PDGF mRNA by adhering to a surface.\textsuperscript{17} Actin microfilament integrity is necessary for the PDGF mRNA expression. Thus, monocytes may become primed in a wound site to produce PDGF, a growth factor critical for the fibroproliferative response, by the very act of diapedesis.

Thus, substances from platelets, the coagulation system, and injured cells not only promote vasoregulation, blood clotting and tissue degradation, but also stimulate processes important for new tissue formation such as cell migration and proliferation and neomatrix formation. Since macrophages can continually produce these factors but platelets cannot, macrophages are pivotal in the transition between wound inflammation and new tissue formation. Prolongation of the inflammatory phase, however, may cause the development of fibrosis manifested as hypertrophic scars, keloids or sclerosis in the skin, and interstitial fibrosis, fibrosing alveolitis, or obliterative bronchiolitis in the lung.

**NEW TISSUE FORMATION**

Repair of most ectodermally derived tissue, like the skin and the lung, entails regeneration of destroyed epithelium and stroma. If extensive or persistent destruction occurs, the original tissue architecture will be obliterated and replaced with scar tissue. In either case granulation tissue first invades the site of damage. The granulation tissue is comprised of macrophages, fibroblasts, neomatrix, and neovascularization which together grow into the wound to form a loosely woven tissue that provides support for the neoeptithelium and scaffolding for the neostroma or scar.

Granulation tissue formation begins when, after a lag of several days, fibroblasts migrate into the wound together with new blood vessel sprouts. TGF-\(\beta\), PDGF, and fragments of matrix proteins such as fibronectin and collagen promote fibroblast proliferation, while FGF and PDGF stimulate fibroblast proliferation.\textsuperscript{18} Once fibroblasts have migrated into the wound, they produce and deposit exuberant quantities of fibronectin, types I and III collagen, and hyaluronate. TGF-\(\beta\) is currently believed to be the most important stimulus of fibroblast ECM production.\textsuperscript{7}

TGF-\(\beta\) also induces fibroblast surface membrane expression of collagen and fibronectin receptors, which are in the $\beta_1$ integrin family (Table 1).\textsuperscript{19} Subsequently, fibroblasts link up with each other and the ECM in radial arrays across the wound, presumably using collagen and fibronectin receptors to "grasp" the ECM.\textsuperscript{14} They develop thick actin cables along their longitudinal axes and generate tension across the wound, causing tissue contraction. PDGF, and perhaps TGF-\(\beta\), stimulate the fibroblasts to contract.\textsuperscript{20} These processes are clearly adaptive in gaping wounds since they shrink the defect. However, in diffuse lung injury the same fibrocontractive processes become maladaptive by reducing total lung volume, by restricting pulmonary expansion during breathing, and by reducing the capacity of gas exchange.

*Table 1—The Integrin Superfamily of Receptors*

<table>
<thead>
<tr>
<th>Receptor Function</th>
<th>Integrin Nomenclature</th>
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<tbody>
<tr>
<td>Laminin binding</td>
<td>$\alpha\beta_1$</td>
</tr>
<tr>
<td>Collagen binding</td>
<td>$\alpha\beta_1$</td>
</tr>
<tr>
<td>Generic ECM binding</td>
<td>$\alpha\beta_1$</td>
</tr>
<tr>
<td>Lymphocyte homing</td>
<td>$\alpha\beta_1$</td>
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<tr>
<td>Fibronectin binding</td>
<td>$\alpha\beta_1$</td>
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<tr>
<td>Laminin binding</td>
<td>$\alpha\beta_1$</td>
</tr>
<tr>
<td>Leukocyte adhesion (MAC-1)</td>
<td>$\alpha\beta_2$</td>
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<tr>
<td>Leukocyte adhesion (LFA-1)</td>
<td>$\alpha\beta_2$</td>
</tr>
<tr>
<td>Leukocyte adhesion (p150)</td>
<td>$\alpha\beta_2$</td>
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<tr>
<td>Platelet binding to ECM</td>
<td>$\alpha\beta_3$</td>
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<td>Vitronectin binding</td>
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Fibroblasts produce a variety of growth factors similar to macrophages; thus, once activated appropriately, fibroblasts may be able to sustain their own growth and/or ECM production. This possibility may have great import in fibrocontractive diseases such as scleroderma, cirrhosis, and pulmonary fibrosis. Collagen matrix appears to have a marked negative feedback on collagen production by normal cultured fibroblasts, and some evidence exists that this may be true also for wound fibroblasts (Jeff Davidson, personal communication).

As mentioned above, new blood vessels form (angiogenesis) concomitant with ingrowth of fibroblasts and neomatrix deposition (fibroplasia). Morphologically, a defined series of events occurs that results in angiogenesis. Endothelial cells lining the microvasculature adjacent to a wound dissolve the intervening basement membrane and emigrate through the disrupted matrix. Endothelial cells migrate into the newly forming granulation tissue as a cord of cells surrounded by a specialized provisional matrix. Adjacent endothelial cell cords join together, forming arcades of new capillaries. Lumina appear within the center of the arched cords and blood flow begins. Basement membrane rapidly forms between the capillary endothelial cells and granulation tissue neomatrix and replaces the specialized provisional matrix from the wound margin inward toward the tips of the capillary sprouts.

FGF is partly responsible for angiogenesis through initiating a cascade of events. FGF stimulates endothelial cells to secrete procollagenase and plasminogen activator. The latter enzymatically converts extravasated blood plasminogen to plasmin. Plasmin as well as PA activates procollagenase to collagenase. Together these enzymes can digest the blood vessel basement membrane. Endothelial chemotactants, such as fibronectin fragments generated from ECM degradation and heparin released from mast cells, draw endothelial cells through the disrupted basement membrane to form nascent capillary bud.

Reepithelialization proceeds rapidly after epithelial disruption, thereby reestablishing the boundary to the outside world. In the skin, reepithelialization begins within hours after injury. Epidermal cells move from the wound margin and residual hair follicles over the wound surface. Motility of epidermal cells across the wound surface is dependent on an epidermal cell metabolism. The alteration of cell phenotype includes retraction of intracellular keratin filaments, dissolution of intercellular desmosomes (structures that normally interlink epidermal cells conferring tensile strength upon the normal epidermis), and formation of peripheral cytoplasmic actin filaments to provide a motor apparatus. Within a day or two, epidermal cells at the wound margin begin to proliferate, providing the migrating epidermis with a new supply of cells. EGF or TGF-α likely provide the signal for this proliferation. Migrating epidermal cells transit over a provisional matrix containing fibrin and fibronectin. Epidermal cells have the capacity to deposit fibronectin in the ECM in vivo. TGF-β may stimulate the cells to do so, and thereby promote translocation over the surface of the wound.

As reepithelialization is established, a new basement membrane forms from the wound margin inward, zippering the new epidermis to the underlying matrix. Once a new stratified epidermis covers the wound surface the epidermal cells revert to their normal function. Tissue repair and reepithelialization of alveoli and airways probably follow a similar sequence of events. However, when excessive tissue damage occurs in the lung, re-epithelialization is delayed and granulation tissue fills the air spaces.

Tissue Remodeling

Tissue remodeling is the final stage of wound repair but greatly overlaps the preceding stage of tissue formation. In fact, ECM remodeling as manifested by the reestablishment of basement membranes begins shortly after granulation tissue formation and reepithelialization. ECM remodeling also occurs within the interstitial tissue. Most fibronectin is eliminated within a week or two after granulation tissue is established. Hyaluronate is replaced and/or supplemented with heparan sulfate proteoglycans in basement membrane regions and with dermatan/chondroitin sulfate proteoglycans in the interstitium. Type I collagen fibers are slowly remodeled to contain less type III collagen and to form large bundles that provide the residual collagen with increasing tensile strength. As a result of ECM remodeling, the easily traumatized granulation tissue slowly evolves into a strong mature scar.

A strong scar limited to an area of traumatic injury is adaptive; however, extensive scarring after widespread skin injury (scleroderma or burns) or widespread lung injury (interstitial fibrosis, sclerosing alveolitis, or obliterator bronchiolitis) is maladaptive. It is hoped that advances in the understanding of wound repair processes in the skin and the lung will lead to the ability to modulate the maladaptive response of these organs.

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