Tissue Engineering

Regulating the Wound Healing Response

In 1883, Elie Metchnikoff had taken up residence with his family in a home overlooking the Mediterranean in the Calabria region of southern Italy. Working in his home laboratory on a day when his family was away at the circus, Metchnikoff made a seminal observation that had implications for the field of immunology, then in its infancy, and for the modern disciplines of tissue engineering and biomaterials as well.

Because they are transparent, starfish larvae are an excellent model system in which to observe cell function. Inserting a rose thorn into a starfish larva, Metchnikoff observed a robust response of wandering cells, which rapidly surrounded the thorn but were unable to ingest it. The wandering cells could internalize smaller particles, as well as infectious microorganisms, and Metchnikoff named them phagocytes.

One type of phagocyte, the macrophage, orchestrates wound healing and the rejection of foreign materials in mammalian tissue. The physical and chemical properties of the foreign material are important in determining the type of macrophage response that occurs.

Around fabrics or porous materials with rough surfaces, macrophages fuse into multinucleate "giant cells" that persist chronically, the so-called foreign body reaction. When they encounter a material with a smooth surface, however, macrophages form a collagen-rich capsule, a process that can be considered a type of fibrosis, and fewer giant cells are evident.

What determines the plasticity of the macrophage response is not well understood. Knowing the range and regulation of this cellular variability will provide new avenues to controlling the formation of giant cells and the extent of fibrosis. Although differences in response exist between organisms, it is increasingly clear that fundamental processes such as immunity and inflammation are highly conserved across phyla. Examining the role of the macrophage in wound healing in diverse organisms and settings will likely prove instructive.

Wound healing occurs in a stereotypic pattern of overlapping stages. Extravasation of blood proteins such as fibrinogen, vitronectin, and fibronectin begins at the instant of injury. These proteins are incorporated into a specialized but transient form of extracellular matrix called the provisional matrix. Hemorrhage accounts for plasma protein leakage during the first few minutes after injury, but extravasation continues for several days as a consequence of an active and specifically regulated increase in vascular permeability.

Concomitant with blood clotting is the appearance within the wound bed of growth factors, cytokines, and additional extracellular matrix proteins, all of which are products of activated platelets. The combination of growth factors and cytokines in the context of the provisional matrix provides important signals to the waves of cells that subsequently immigrate into the wound. Rather than serving as a passive scaffold for cell migration, the provisional matrix is actively involved in determining cell behavior during wound healing. It is also recognized that the spectrum of proteins that bind to implanted, engineered devices determines, in part, the ensuing tissue response to the device.

Within minutes of injury, the inflammatory phase of wound healing begins, and a type of phagocyte called a neutrophil appears at the wound site. Neutrophils roll on the vascular endothelial surface, pass through the vessel wall, and move into the site of injury. A considerable body of knowledge now exists indicating a role for endothelial cell surface proteins called selectins in the rolling process. Extracellular matrix proteins and cell surface receptors including integrins mediate the firm binding of neutrophils to vessel walls and then their passage into tissue.

The function of neutrophils in the healing wound is primarily to sterilize the wound through processes involving both phagocytosis and release of oxygen radicals. To carry out phagocytosis, neutrophils bind avidly to opsonins such as the components of complement, which normally coat bacteria.

Coincident with the appearance of neutrophils in a wound is the arrival of monocytes, which are initially fewer in number than neutrophils. In the first few days after injury, monocyte numbers increase markedly, and monocytes in the site of injury differentiate into macrophages. These remove dead neutrophils, digest microorganisms, and degrade and phagocytose devitalized tissue components.

Macrophages are also the source of a wide range of secreted mediators: growth factors (such as TGF-β, PDGF, IGF-I, and FGF's), cytokines (CSF, TGF-α, IL-1), and extracellular matrix proteins. Macrophage products are thought to be critical in coordinating the events that occur in healing wounds, as well as events surrounding implanted biomaterials.

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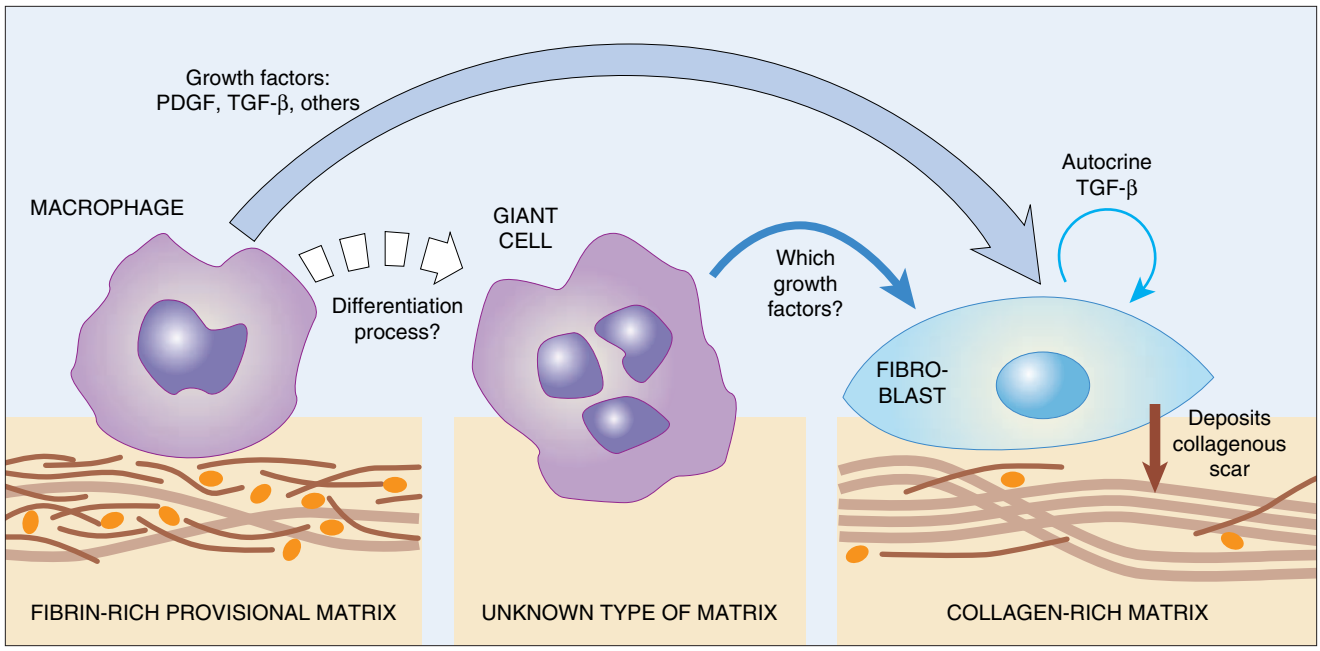

The hypothesized collaboration between macrophages and fibroblasts involves macrophage-derived growth factors and the extracellular matrix context in which the cells are embedded. Fibroblasts can also undergo autocrine stimulation by TGF-β. The mechanisms that control the formation of multinucleated giant cells and their effects on fibroblasts are unknown.

As Metchnikoff found, macrophages are phagocytic cells. However, when they encounter a particle that dwarfs them, they undergo a process that has been termed "frustrated phagocytosis." They may remain chronically activated and secrete either more of certain growth factors, cytokines, and matrix proteins or a different spectrum of these products. The mechanisms that regulate this process are unknown.

In healing wounds and around some types of engineered tissues, macrophages, fibroblasts, and capillary sprouts appear as a unit by about four days after injury (or implant). Robust growth of new vessels is observed, and the result is granulation tissue. Neovascularization is driven by extracellular matrix proteins, growth factors, proteases, and other factors.

Around implanted biomaterials as well as in healing wounds, macrophages and fibroblasts likely collaborate in stimulating mesenchymal cell proliferation and tissue remodeling. This process, called fibroplasia, results in the deposition of scar tissue. The scar, rich in collagen, fibronectin, elastin, and proteoglycans, is deposited in minimal amounts in normal wounds but in excessive amounts around certain biomaterials.

Movement of fibroblasts into a wound is impelled by chemotactic factors that include PDGF, TGF-β, C5a, and fragments of matrix proteins. The extracellular matrix modulates the spectrum of responses to the growth factors. Thus, when embedded in fibrin, fibroblasts stimulated by PDGF express more fibrin receptors. When embedded in collagen and in the presence of PDGF, fibroblasts elaborate more collagen receptors.

If the collaboration between macrophages and fibroblasts is such an intimate and reciprocal one, then what are the factors that control it? What are the circumstances during a close encounter with a biomaterial that lead macrophages to differentiate into giant cells or alternatively into resting macrophages? These questions have begun to be addressed.

It will be important to learn which cellular receptors and intracellular signaling networks control macrophage differentiation pathways. Clearly, gaining control of the differentiation process would have an amplifying effect, influencing fibroblast function and as a consequence the extent of fibrosis around engineered tissues and implanted biomaterials.

At present, TGF-β_1 is known to be a key regulator of fibroplasia, and a number of strategies are being used to modulate the function of TGF-β_1 in vivo. Some of these strategies are in clinical trials and may be useful as therapies for fibrosis. However, they may also have efficacy in regulating the host response to implanted devices.

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