Commentary: Engineering of Tissue Healing and Regeneration

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ABSTRACT

Regenerative medicine aims to restore homeostasis of diseased tissues and organs. With time, engineered replacement tissue constructs will play an increasingly important role in achieving this goal. Equally important, however, will be the ability to resolve disease-associated inflammation and to optimize tissue regenerative capacity by specifically patterning the host tissue microenvironment. The tools of bioengineering are uniquely suited to meet these challenges. Here, the candidate molecular and cellular targets for manipulating the host's inflammatory environment and tissue regenerative capacity are briefly discussed within the context of current and emerging bioengineering strategies. The objective is to draw the attention of basic scientists and engineers to the importance of regulating inflammation in achieving the goals of tissue engineering and regenerative medicine.

INTRODUCTION

NOTABLE STRIDES have recently been made in developing biodegradable, low-toxicity materials that can mimic the natural cellular microenvironment. These materials can be programmed to exhibit desired properties, such as mechanical strength, malleability, extracellular matrix (ECM)-like characteristics, and bioactive ligand presentation.[1–4] New 3-dimensional material and cell fabrication techniques are now being developed using these materials and stem cell populations for generation of 3-dimensional organ-like constructs *in vitro*.[5,6] These technological achievements, informed by ever-advancing knowledge of cell and developmental biology, will facilitate the design and fabrication of replacement tissues and organs. However, for successful clinical translation of tissue-engineering methodologies, it is also critical to ensure proper *in vivo* function of engineered constructs under microenvironmental conditions of inflammation and fibrosis, which typically characterize damaged tissues. Recent progress in understanding the mechanisms of inflammation resolution[7,8] opens new possibilities for rational control of the tissue inflammatory microenvironment.

Also of significance to the future of tissue engineering therapies are recent works suggesting that specific patterning of the inflammatory response may play a role in augmenting scarless wound healing and even in enhancing the regenerative capacity of mammalian tissues.[9–13] Thus, it will be important to derive sophisticated strategies for promoting inflammation resolution while enhancing tissue regeneration. Rather than simply blocking the inflammatory response, these strategies should aim at temporal and spatial control of the inflammatory tissue microenvironment. It is hypothesized that tools of modern bioengineering can offer a powerful means for engineering this sophisticated control.

RESOLUTION OF INFLAMMATION: AN ORCHESTRATED SEQUENCE OF EVENTS

Acute inflammation resulting from infection, tissue injury, or surgical trauma can resolve, returning the tissue to its normal physiological state, or it can evolve into a chronic inflammatory condition characterized by continuous tissue destruction, fibrosis, and scarring.[14] Important insights have recently been made into the mechanisms of acute inflammation and its resolution. It was found, for example, that resolution of inflammation is a highly active, temporally and spatially coordinated process controlled by endogenous "pro-resolving" mediators.[8,15,16] These mediators have become

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the focus of intense investigation as promising molecular targets for new drug discovery to augment acute inflammation resolution and to combat chronic inflammatory diseases.[7] Unlike the traditional anti-inflammatory drugs, which block initiation of inflammation, the emerging "pro-resolving" drugs are designed to mimic physiological resolution of inflammation.

Briefly, acute inflammation begins with an influx of neutrophils and other leukocytes to the site of infection or tissue damage (Fig. 1).[7,8] These cells are necessary during the early phase of inflammation, but if not cleared in time, they can damage healthy tissue through generation of free radicals and other mechanisms. The production of pro-inflammatory cytokines and cell adhesion molecules, as well as of prostaglandins and leukotrienes, which are essential for vascular dilation, the increase of blood flow to the wound, and lymphocyte trafficking from the circulation into the interstitial space of the tissues, also characterize the early phase of acute inflammation. Prostaglandins and leukotrienes work as "resolution switches" by inducing production of inflammation-resolving lipid mediators: lipoxins, resolvins, and protectins, which are responsible for attracting monocytes and macrophages to the site of tissue damage, stimulating

macrophages to clear apoptotic neutrophils, retarding the entry of new neutrophils to the site of inflammation, and reducing vascular permeability. Collectively, these events lead to the restoration of normal physiological function of the tissue. Among other mediators of inflammation resolution are caspase proteases, responsible for execution of apoptosis;[17] CD44, macrophage-specific hyaluronan receptor controlling the capacity of macrophages to clear apoptotic neutrophils;[17] and nuclear factor-kappa B(s), the key multi-functional regulators of the mammalian immune response.[18]

TO SCAR OR TO REGENERATE?

Successful inflammation resolution limits tissue injury and prevents development of chronic immune-mediated inflammation. If acute inflammation is not properly resolved, however, it will persist, resulting in a varying degree of tissue injury and the formation of a non-functional fibrotic scar. Tissue destruction will continue, in part because the dead cells maintain a vicious cycle of tissue destruction by stimulating production of pro-inflammatory cytokines via Toll-like receptor–mediated signaling (Fig. 1).[19] This is a common
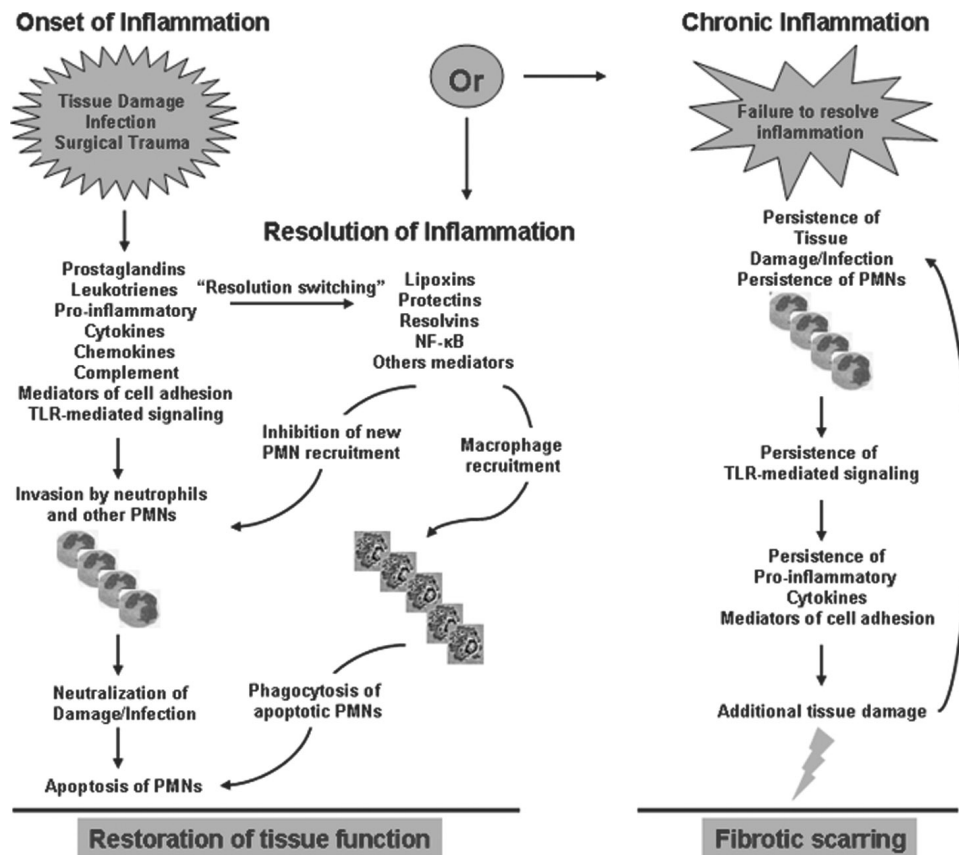

FIG. 1. Simplified schematic representation of resolution of inflammation leading to restoration of tissue function, and of chronic inflammation leading to fibrotic scarring. See text for additional explanations.

outcome of tissue injury in mammals, and it is crucially important to take this reality into account when designing future tissue engineering strategies. In this regard, it is important to study systems that can support productive tissue regeneration– (i.e., systems in which inflammation is effectively resolved and tissue function is restored). For example, regeneration of selected embryonic tissues[20,21] and of the adult liver[22] and gingiva[23] have been documented. Also, the immunologically compromised MRL mouse can re-grow adult cartilage, skin, hair follicles, and heart myocardium with minimal inflammation and scarring,[24,25] and the PU.1 null mouse that lacks functional neutrophils and macrophages exhibits a fetal-like pattern of wound repair.[26,27] Moreover, unlike mammals, fish and amphibians can successfully regenerate a variety of different tissues and organs without scarring.[28]

The emerging evidence suggests that similar to inflammation resolution,[8,15] productive tissue regeneration may also result from a precise combinatorial, temporal, and spatial orchestration of the inflammatory response to tissue damage.[10] For instance, the results of gene expression profiling of zebra fish heart and fin regeneration suggest that a specific temporal order of expression of wound healing genes (such as tenascin C, cathepsin B, and others), in addition to the growth factor genes and tissue remodeling genes, may be important for a productive regenerative response.[29,30] In the mouse liver, coincident signaling of two pro-inflammatory cytokines (interleukin-6 and tumor necrosis factor-α (TNF-α)) primes regeneration, although TNF-α alone elicits liver scarring.[31] Furthermore, the outcome of mouse skin regeneration varies dramatically depending on the particular transforming growth factor-β (TGF-β) isoform and the timing of TGF-β application to the wound.[21] Collectively, these findings suggest that it may be possible to diminish or avoid scarring via directed patterning of the wound microenvironment in time and space.

Several recent, compelling studies provided evidence that immune mediators may play functional roles in the stem cell microenvironment called the stem cell niche.[32] For example, in the mouse central nervous system (CNS), signaling of pro-resolving mediators of acute inflammation, leukotriene B_4 and lipoxin A_4, control proliferation and differentiation of neural stem cells.[12] Also in the CNS, the macrophage-secreted calcium-binding protein oncomodulin promotes regeneration of the optic nerve,[9] and macrophages transplanted into an injured spinal cord facilitate its functional recovery.[33] In the mouse colon, macrophage activation mediated by gut microbiota is required for the amplification of colonic epithelial progenitors, which occurs in response to colon damage.[11] Still another example is that of mouse skin- and lung-resident dendritic epidermal T cells ($\gamma\delta$T) cells, which substantially accelerate epidermal and lung wound healing by producing growth factors that control progenitor cell proliferation.[34,35]

The novel function of the complement cascade in wound healing and regeneration is also of much interest. Although traditionally considered solely as an effector system in the host defense against invading pathogens and other insults, more-recent studies support a general role of complement in early development, stem cell proliferation and differentiation, and tissue regeneration.[36–38] It has been proposed that the complement cascade might have evolved as an important immune ''partner'' that communicates acute tissue damage or stress to the tissue regeneration machinery.[36]

In light of these new, intriguing findings, it is tempting to speculate that the inflammatory response may play a role in tissue regeneration (e.g., in controlling the function of stem and progenitor cells) that is distinct from its well-recognized role in wound healing. It is possible to envision that, by ''fine tuning'' of the immune response, we may not only be able to achieve a scarless wound healing, but may also enhance the normally limited regenerative capacity of mammalian tissues. Given the complexity of the inflammatory response, it is unlikely that such ''fine tuning'' can be achieved using traditional therapeutic modalities. On the other hand, the tools of modern bioengineering, with their power to predictably pattern tissue microenvironment, have tremendous potential for contributing to this goal.

ENVIRONMENT BY DESIGN

The last several years have brought significant progress in design and fabrication of biomaterials, creating new opportunities for mimicking and modification of the tissue microenvironment and the immune response. Important examples of this progress include cell-instructive ECM-like materials that can communicate multiple regulatory signals to the tissues in a temporally and spatially defined fashion and display specific cell-adhesive and homing signals, smart self-assembling biomaterials that can alter their properties in response to external stimuli, and material fabrication technologies that allow creation of tissue-like structures with defined 3-dimensional architectures mimicking normal tissue organization. Because this work has been extensively reviewed recently,[2–5,39–42] some notable methodologies of potential relevance to the patterning of the inflammatory response will only be briefly highlighted.

David Mooney's group has developed a method for temporal *in vivo* delivery of multiple growth factors.[43,44] This method uses polymeric scaffolds fabricated from poly(lactide-co-glycolide) (PLG). The growth factors, vascular endothelial growth factor (VEGF) and platelet-derived growth factor (PDGF), are incorporated into the scaffold using 2 methods to regulate their rate of release; for rapid release, VEGF is mixed with the PLG particles before the scaffold fabrication, whereas for slower release, PDGF is pre-encapsulated into PLG microspheres. In the homogeneous scaffold obtained from the fusion of the particulate and the microsphere PLG, the magnitude of the factor release can be readily adjusted by altering the amounts of the

factors incorporated into the scaffold, whereas the rate of the release can be varied by changing the degradation rates of PLG. The investigators have shown that VEGF and PDGF delivered *in vivo* using this method are more effective in enhancing angiogenesis than bolus delivery. Although these types of strategies have been used exclusively for growth factor delivery, it should be possible to adapt them for the delivery of the modifiers of the immune response, such as the "pro-resolving" drugs and the cytokines, to be released at defined time points during inflammation progression.

Dendrimers, which are polymeric branched molecules composed of multiple branched monomers, also represent promising cell-instructive materials for delivery of multiple biomolecules.[4] A valuable property of dendrimers is that they can be simultaneously modified with different ligands that can be displayed at precisely defined ratios. An interesting example of using dendrimers as cell-instructive vehicles is the simultaneous delivery of angiostatin and tissue inhibitor of metalloproteinase-2 genes to mouse tumors, resulting in a drastic inhibition of tumor-associated vascularization.[45]

Jeffrey Hubbell's laboratory has developed synthetic ECM-like biointeractive poly(ethylene glycol) (PEG)-based hydrogels that release growth factors in a spatially controlled fashion (i.e., only upon local cellular demand).[46,47] The PEG networks contain a combination of pendant oligopeptide ligands for cell adhesion and substrates for matrix metalloproteinases (MMPs). The networks also incorporate growth factors; in the two cited works, VEGF and bone morphogenetic protein-2 (BMP-2) were employed, but the system can, in principle, accommodate other active biomolecules. Moreover, by altering the functionality and molecular weight of the PEG building blocks, the internal architecture of the hydrogel can be tailored to specific applications. Migratory cells, such as fibroblasts, can migrate into these bioactive networks *in vitro* and *in vivo*. Because these hydrogels display MMP recognition sites, they undergo cell-mediated proteolytic degradation, thereby triggering local growth factor release. The investigators showed that, when implanted into critical-size cranial defects in a rat model, hydrogels containing BMP-2 were completely infiltrated with cells and were remodeled into bone-like tissue.[47] The VEGF-containing hydrogels were remodeled into a vascularized tissue.[46] One can envision the use of such biointeractive hydrogels in orchestrating inflammation resolution. For example, by taking advantage of the capacity of these hydrogels to release the biological modifiers upon a local cellular demand, it may be possible to design them to release the "pro-resolving" drugs, cytokines, and other bioactive molecules at the right time and in the right place and in response to the signals from the surrounding cells (e.g., to direct monocytes and macrophages to the site of injury to clear apoptotic neutrophils; see above, also Fig. 1). Such strategies may help to overcome chronic inflammation, progressive tissue injury, and scarring.

Samuel Stupp and his co-workers have made an important advance in development of smart self-assembling biomaterials. The group has developed synthetic amphiphilic peptides that can form aqueous solutions but can also be triggered to self-assemble into ECM-like nano-fiber networks using a pH change or upon injection *in vivo*.[48–50] The nanofibers can be customized for a specific cellular response through modification of the peptide–amphophile sequence. It has been shown that the scaffolds formed by such nanofibers can direct mineralization of hydroxyapatite to form a composite material structurally reminiscent of bone.[48] The nanofibers modified to display neurogenesis-promoting laminin epitope can direct *in vivo* neuronal differentiation of neural progenitor cells while suppressing astrocyte differentiation.[49] When the nanofibers were self-assembled in the presence of heparin bound to fibroblast growth factor-2 and VEGF, they strongly promoted angiogenesis *in vivo*.[50] Because these versatile peptide-amphophiles can be custom designed to elicit specific cellular responses, they are likely to find multiple uses for inflammation resolution and tissue regeneration applications. An additional advantage of these compounds is that they can be specifically delivered in liquid form to the site of tissue damage by simple injection.

Recent advances in computer-aided design algorithms and rapid prototyping (also known as solid free-form fabrication) make it possible to create scaffold constructs with highly predictable shapes and internal architecture.[5,6] The method consists of manufacturing 3-dimensional scaffold prototypes using an additive process, in a layer-by-layer fashion, based on a computer representation of the prototypes' topological parameters. The additive nature of the rapid prototyping process allows generation of scaffolds with defined structure parameters, such as pore size, shape, and connectivity. More recently, the concept of layered manufacturing has been extended to "organ printing," which allows production of organ-like constructs.[51,52] In the future, these and other approaches that rely on principles of cellular self-assembly into tissues[53] should make it feasible to produce vascularized and innervated organs. Such "organ printing" methodologies could also be used for predictable patterning of the microenvironment of the engineered organs. For example, it should be possible to attain the specific spatial organization of the ECM and other bioactive molecules within the engineered organs to enhance their biocompatibility and functional integration into the host tissues.

CONCLUSIONS

We are beginning to understand the mechanisms of inflammatory resolution, chronic inflammation, and wound healing. Progress in this area opens exciting opportunities for rational control of these processes, which are central to treatment of the diseases of virtually every human tissue and organ. Moreover, the growing evidence that the immune

system may play a positive role in tissue regeneration provides an additional incentive for controlling the inflammatory response. For example, with regard to treatment of neurodegenerative diseases, the following has been stated:[13] "Since many of these responses (inflammatory) can exert potent beneficial effects, directing and instructing the inflammatory machinery may be a better therapeutic objective than suppressing it." It is unlikely that traditional anti-inflammatory drugs that block the initiation of inflammation or the systemic delivery of these drugs will be able to effectively instruct and direct the inflammatory machinery for achieving the needed therapeutic objectives. In contrast, the continually evolving tools of bioengineering, with their ability to shape the cell-instructive structurally defined microenvironment that can react to external and internal stimuli have great potential to predictably orchestrate the inflammatory response.

Most adult mammalian tissues appear to have a limited regenerative capacity. Tissue regeneration, however, is normally evaluated within the context of the ongoing acute or chronic inflammatory processes, which could potentially mask or directly inhibit tissue regeneration. One can envision that, if we learn to control the destructive forces of inflammation, we might reveal and even enhance the regenerative capacity of human tissues. Although still in need of definitive proof, the evidence in support of this scenario is accumulating. For example, it appears that active CNS and pancreatic β-cell regeneration may take place in patients with multiple sclerosis and type 1 diabetes, respectively.[54,55] It has been proposed that, in these patients, the net result is tissue degeneration, because the relentless autoimmune-mediated tissue destruction masks the regeneration. If we could learn how to harness the immune-mediated tissue destruction and scarring and to optimize regeneration, the current models of tissue engineering (relying on tissue reconstruction, largely from exogenous cells and biomaterials) might evolve into models of true regenerative medicine in which tissues will be tweaked to efficiently heal themselves.

ACKNOWLEDGMENTS

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