Trauma-Induced Inflammation and Fracture Healing

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Summary: Fracture healing is an extremely complex interaction of cells, biologic pathways, and molecules. Certainly, the inflammatory response is one of the initiating factors for bone healing. The inflammatory phase is a critical period characterized by low oxygen tension, impaired perfusion, and the migration of a wide array of cells and release of active molecules. Systemwide inflammatory conditions also modulate the primary processes of fracture management. Osteoprogenitor cells, mesenchymal cells, osteoblasts, and chondrocytes contribute to the healing and inflammatory response at the bone level. The inflammatory process is dependent on and propagates through proinflammatory cytokines, the transforming growth factor-β superfamily with other growth factors, and the metalloproteinases and angiogenic factors. Interference with any of these pathways or proteins either promotes or more likely decreases fracture healing. This article reviews the initial inflammatory response to trauma as it pertains to musculoskeletal healing.

Key Words: fracture, inflammation, stem cells, bone, healing (J Orthop Trauma 2010;24:522–525)

INTRODUCTION

Fracture healing is still poorly understood. Healing is an extremely complex interaction of cells, biologic pathways, and molecules that temporally and spatially interact to produce a response to bone fracture. Initial hematoma formation is followed by inflammation, repair, and finally, remodeling. Certainly, the inflammatory response is one of the initiating factors for bone healing and inflammation carries through even into the hard callus formation. The inflammatory phase is a critical period characterized by low oxygen tension, impaired perfusion, and the migration of a wide array of growth factors. Systemwide inflammatory conditions also modulate the primary processes of fracture management. Osteoprogenitor cells, mesenchymal cells, osteoblasts, and chondrocytes1 all contribute to the healing and inflammatory response at the bone level. Platelet-derived growth factor, transforming growth factor-β (TGF-β), and interleukin (IL) 1 and 6 and prostaglandins are among the key group of inflammatory chemokines that initiate repair through their effects on the marrow, periosteum, and hematoma. The inflammatory process continues through three groups of promoting molecules: the proinflammatory cytokines, the TGF-β superfamily with other growth factors, and the metalloproteinases and angiogenic factors.2 The TGF-β superfamily is more critical to the postinflammatory response and, although not discussed in depth in this article, does have implications for the inflammatory response. Interference with any of these pathways or proteins either promotes or more likely decreases fracture healing.

Initial Inflammatory Response

The inflammatory response is in part the result of both the local tissue injury and the immunologic reaction that is caused by local necrosis, bacterial ingress, and hypoxia. Two distinct arms of the immune system are mobilized in response to injury. Inflammation is triggered when the body detects the presence of invading substances. The innate immune system is mediated primarily by macrophages and neutrophils that provide the first defense against invaders and injury. These cells kill bacteria, phagocytose debris, and stimulate production of granulation tissue. In contrast, the adaptive immune system is mediated primarily by T-cells and B-cells. These cells provide protection to specific pathogens. Their “memory” provides a rapid response when specific pathogens are encountered subsequently. Together these two arms of the immune system produce the inflammatory response that provides host defense in disease states, including injury.

Inflammatory Cells During Fracture Healing

The contributing factors at the initial inflammatory phase include cytokines, platelets, bone morphogenetic proteins, and mesenchymal stem cells. Polymorphonuclear leukocytes (PMNs) represent 50% to 60% of total circulating leukocytes and are the leading cells in the first response to severe trauma from the incident on up to the second or third day. PMNs at the site of trauma are important for wound healing and protection against infection. However, PMN activation can lead to involvement of organs not primarily affected by the initial trauma.3 Activated PMNs recruited into other organs are a main source of secondary organ and tissue damage.

IL-1, IL-6 and TNF-α are secreted by the inflammatory cells, including PMNs. These factors have a chemotactic effect on other inflammatory cells and on the recruitment of mesenchymal cells. The interleukin inflammatory response seems to be a transitory response. It has been reported that expressions of IL-1 and IL-6 spike 1 day after fracture followed by a rapid decline to near undetectable levels by the
third day postinjury. A close relationship between inflammation and soft tissue responses to fight infection is established at the same time. Undifferentiated monocytes reside in the effective reservoir of the spleen and outnumber the equivalents in the circulation. Local production of chemokines leads to recruitment of immune cells, including these monocytes, which initiate the body’s host defense system. A series of chemokine ligands, including CCL2, CCL7, CCL8, and CCL13, recruit macrophages and other cell types to sites of injury. These molecules activate receptors such as CCR2 and MSR1 on inflammatory cells and stimulate release of cells from the bone marrow and recruitment of cells from the circulatory system. Immediately after injury, tissues at the fracture site produce high levels of CCL2, CCL7, CCL8, and CCR2 and MSR1. At this time, macrophages and neutrophils, among other inflammatory cell types, are present at the fracture site. The inflammatory response is believed to have both positive and negative effects on bone repair, but the underlying cellular mechanisms are not well understood. For example, the lack of cyclo-oxygenase-2 (COX-2), a target of nonsteroidal anti-inflammatory drugs (NSAIDs), inhibits osteoblast differentiation in the periostium. In contrast, transplanted mesenchymal stem cells, which can improve healing, have systemic anti-inflammatory effects on the cytokines released after fracture, including TNF-α and IL1-β. Inflammatory mediators such as TNF-α can also impair later stages of repair by stimulating cartilage degradation.

The role of macrophages during fracture healing was examined for fracture repair in mice that lacked the CCR2 gene. There is a decreased number of macrophages at the fracture site, decreased surface density of blood vessels, and alterations in callus parameters. Some of these changes resulted from delays in bone formation, whereas other alterations were the result of impaired callus remodeling. Overall, the results illustrate the necessity for monocytes during early and late stages of fracture healing.

Simultaneously, platelets activated by thrombin and local exposed collagen release platelet derived growth factor and TGF-β that among others also play a part in initiating fracture repair. These factors combine to induce angiogenesis, chemotaxis of acute inflammatory and mesenchymal cells, and further platelet aggregation. The platelets then continue to act in a positive feedback loop, until interrupted, as drivers of cellular and protein aggregation and activation. Concurrently, bone morphogenetic proteins are released from the bone matrix and expressed by recruited primary mesenchymal cells. Mesenchymal stem cells then proliferate and differentiate down either a chondrogenic or osteogenic lineage depending on local biochemical and biomechanical cues.

Despite the evolving comprehension of more of the active ingredients, the overall effects of systemic and local inflammation on the bone healing response are virtually unknown. On the local level, studies on fracture hematoma have clearly demonstrated that it is different from muscle hematoma. A fracture hematoma contains a lower ratio of leukocytes, higher ratio of necrotic cells, and a higher ratio of T-cells and may therefore react differently in terms of inflammation and healing. The physiological triggers that result in differential cellular expression are not known. Therefore, stimuli that may be systemic or originating in other organs than the bone could have an effect on osteogenesis.

In addition to playing a positive role during healing, inflammation is also associated with perturbations to healing. In particular, age-related changes in the inflammatory system are associated with decreased healing potential. Authors have shown that fracture healing is delayed in old animals. The extent to which juvenile inflammatory cells could restore healing in aged animals has been tested by transplanting juvenile bone marrow into lethally irradiated aged mice. In this model, the transplanted cells only contribute inflammatory cell types to the fracture callus. All of the bone and cartilage tissues are produced by host cells. The chimeric animals had reduced serum levels of IL-6 and TNF-α that were similar to juvenile animals. Observed accelerated fracture healing in the chimeric animals suggested that rejuvenation of the inflammatory system stimulated repair in the aged animals.

Matrix Metalloproteinases and the Cellular Response to Inflammation After Skeletal Injury

Matrix metalloproteinases (MMPs) are integral to the inflammatory response and cell differentiation at the fracture site. MMPs play important roles in cartilage and bone remodeling and angiogenesis during the soft callus and remodeling phases of repair. They are also expressed in the early stages of repair in various cell types, including inflammatory cells. The lack of MMP-9 leads to healing of stabilized fractures through endochondral ossification, whereas wild-type mice heal through intramembranous ossification. There are differences in the profiles of inflammatory cells in MMP-9 null mice compared with wild-type mice suggesting MMP-9 may regulate cell differentiation by modulating the inflammatory response. To functionally assess the link between the profile of inflammatory cells and cell differentiation in MMP-9 null mice, MMP-9+ mice were transplanted with wild-type bone marrow, which can only provide hematopoietic cells to the fracture site. The authors found that after transplantation, MMP-9+ mice healed stabilized fractures through intramembranous ossification and exhibited a normal profile of inflammatory cells at the fracture site (unpublished observations). These results indicate that MMP-9 in part mediates its effects on cellular differentiation through regulating the inflammatory response. Many other MMP family members may be involved in the recruitment of inflammatory cells, their functions, and their clearance from the fracture site.

Biomechanics of Fracture Repair Affect the Inflammatory Cascade

The biomechanics of fixation have implications on the inflammatory response as well. In rigidly stabilized fractures, the process leads to direct bone formation, whereas in the other case, fracture instability causes cartilage and bone formation. Comparison in mouse models of these two fracture environments has assessed the role of inflammation on skeletal cell differentiation. There are changes in the expression patterns of inflammatory genes and in the recruitment of inflammatory cells. The results indicate that modulations in the inflammatory response in stabilized and nonstabilized fractures may influence the mode of repair.
During early inflammation, angiogenesis takes place, which is a prerequisite for further progression of the regeneration cascade. The vascular ingrowth into the developing callus is regulated by fibroblast growth factor, vascular endothelial growth factor, and angiopoietins. Vascular endothelial growth factor proliferation occurs later than angiopoietin with its greatest effect during endochondral bone formation. Local conditions under which the vascularity can be improved are local tissue factors such as bony stability. It appears that regulation of bone growth appears to be dependent on interactions between various hormones and vascular endothelial growth factor. Angiogenic factor is a mediator for the recruitment of endothelial cells. After their recruitment, endothelial cells undergo a time-dependent gene switch that promotes bone remodeling. Likewise, endothelial cells may act as a source to release mitogens for osteoblasts.

ANTI-INFLAMMATORIES: EFFECTS ON FRACTURE HEALING

Anti-inflammatories are frequently used for systemic disease or to modulate the systemic inflammatory response or just for pain management. COX is the necessary enzyme for conversion of arachidonic acid to prostaglandin-G2. COX-1 is expressed in osteocytes, osteoblasts, and osteoclasts. COX-2 is an inducible enzyme that is abundant in macrophages during inflammatory states. It is also the predominant form of COX in a fracture healing milieu and is critical to enchondral healing. Nonselective NSAIDs (such as ibuprofen, naproxen, and indomethacin among others) block the action of COX-1 and -2. COX-2 inhibitors have been described to cause a dose- and time-dependent impairment of bone formation. Animal studies have defined the role of COX-2 in fracture healing. COX-2 is a stress response gene (seen in inflammatory conditions) that mediates skeletal repair through the induction of osteogenesis. COX-2 knockout mice have normal skeletal development but impaired fracture healing. In a fracture model, COX-2 knockout mice demonstrate reduced chondrogenesis and a dampened proliferative phase. Bone formation is delayed and vascularization of the fracture callus is limited. Evidence from these and other animal studies support the contention that all NSAIDs can delay or inhibit fracture healing in a time-dependent and reversible fashion. This effect stems largely from inhibition of COX-2. Further research has shown that administration of COX-2-specific inhibitors in the immediate postinjury period will delay healing, whereas delaying treatment until 14 days postinjury will not alter healing. Any impact on fracture healing seems to be entirely reversible once treatment is withdrawn. Negative effects on bone healing induced by leukotrienes can be reduced by leukotriene antagonists. Therefore, a cause–effect relationship appears to occur between the suppression of inflammation and bone healing. Ongoing research has revealed numerous inhibitory molecules that regulate different signaling pathways seen not only in bone regeneration, but also in other cellular processes. Evidence from human studies is limited. The majority of studies are retrospective and confounders skew the interpretation of findings. Burd et al showed that patients who received indomethacin as treatment for an acetabular fracture had a greater risk of long-bone nonunion than those who were treated with radiotherapy. Another recent study looked at 9995 humerus fractures in a Medicare database showing a correlation between the use of NSAIDs and nonunions. The conclusion was that patients with nonunions had more pain and thus required pain relief at that late stage. Although there is extensive and compelling evidence in animals that NSAIDs impair fracture healing, the reversibility of this effect as well as the absence of support from the human study literature raises the question of clinical significance. Reconciling the data, one can say that consistent therapeutic doses of NSAIDs given in the immediate postinjury period may result in increased cartilage formation and delayed bony callus formation, but the cumulative effect on clinical healing is uncertain.

Surgery and the Systemic Response

From this discussion, there may be a substantial, yet unknown impact of our surgical strategies and the timing of operations. It appears to be common sense that early fracture fixation is beneficial. Although the reasons to do so have been empiric and clinically based, they also might be correct at the molecular basis of fracture healing. On the systemic level, the early hyperinflammatory response to severe trauma is known to be followed by a hypoinflammatory phase. The biphasic response implies that the number of complications is higher when unexpected events occur during the hypoinflammatory (energy) phase.

The trauma impact (first hit) causes primary organ injuries with local tissue damage as well as an activation of the systemic inflammatory response (early hyperinflammatory phase). There is an established inflammatory cytokine response after injury that involves increased serum levels of interleukins, TNF, and interferon. Complement components C3a and C5a are potent neutrophil chemoattractants and also increase vascular permeability. The presence of early and late multiple organ dysfunction depends on the initial degree of injury severity and extent of shock. Early multiple organ dysfunction is associated with severe injury and shock and is accompanied by an early vigorous proinflammatory response. Late multiple organ dysfunction is often associated with a second hit, particularly during the hypoinflammatory phase. Secondary endogenous and exogenous factors play a crucial role in the initiation and severity of posttraumatic complications. Typical second hits are hypoxia, metabolic acidosis, ischemia, reperfusion, and infections. Surgical interventions that cause severe tissue damage, hypothermia, or blood loss as well as massive transfusions, inadequate, or delayed surgical or intensive care all represent iatrogenic second hits.

SUMMARY

In conclusion, various components of the inflammatory response can either stimulate or compromise healing through regulating cell recruitment and differentiation. The effects of local and, in particular, systemic inflammation may be more substantial than described so far. An early hyperinflammatory local reaction might be related to the initiation and degree of fracture healing. Additionally, other factors such as
interactions with other cascade systems probably play a more important role than currently understood. To clinically improve the management of inflammation after bone injury, more studies will be required to dissect the roles of numerous inflammatory factors and their impact on skeletal cell differentiation.

REFERENCES
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