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MEETING REPORT

Recent insights on the endogenous bone repair mechanisms and therapeutic applications

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Meeting Report from the Annual Meeting of the Orthopaedic Research Society, San Francisco, CA, USA, 4–7 February 2012.

Role of Inflammation in Bone Repair

Over 100 reports related to the biology of fracture repair were presented at the 2012 ORS meeting. With 5–10% of patients exhibiting delayed healing after bone injury, new strategies to enhance repair are highly needed and will rely on a better understanding of the endogenous mechanisms of bone repair. One important area of investigation is the role of the inflammatory response, as inflammation has been shown to be an important regulator of bone regeneration. Using genetic mouse models, several groups have previously reported that the adaptive immune system was detrimental, whereas the innate immune system was favorable to healing.[1–4] Nam *et al.*[5] looked more closely at the effects of T lymphocytes on osteoblasts in RAG$^{-/-}$ mice and found that pro-inflammatory cytokines secreted by T lymphocytes, such as IL-17, can promote osteoblast differentiation. Although the absence of T lymphocytes has a positive impact on later stages of bone repair, osteoblast differentiation during the early stages of repair appears to be improved. Similarly, macrophages have been shown to support osteogenesis but may also be inhibited to stimulate repair in the elderly population as suggested by the ability of PLX3397, an inhibitor of receptor for macrophage-colony stimulating factor activity in macrophages, to rescue delayed healing in aged mice.[6] In a model of systemic inflammation induced by blunt chest trauma in the rat, Recknagel *et al.*[7] further showed that disturbance in the initial inflammatory response causes impaired bone healing. Systemic inflammation was marked by increased serum IL-6 levels and was associated with enhanced infiltration of neutrophils but reduced infiltration of macrophages at the fracture site. This study shows that an imbalance of inflammatory cell recruitment during the initial phase of repair can have severe consequences on late stages of repair, with a decrease in callus and bone volumes and inferior callus mechanical properties. The same authors also reported that systemic inflammation is increased when an external fixator is replaced by an intramedullary nail, a procedure usually performed in polytraumatic patients.[8] In the same rat model of blunt chest trauma, the switch from external to internal fixation further delayed bone repair. Bindl *et al.*[9] reported that severe immune deficiency in Nod-Scid IL-2Rgamma null mice impairs bone healing. In the absence of both innate and adaptive immune systems, all immune cells are affected, leading to decreased callus stiffness, increased cartilage content and decreased osteoclast activity. Altogether, these studies point out the importance of modulating the inflammatory response to treat patients that have sustained traumatic injuries. Understanding the specific roles of distinct inflammatory cell types and cytokines at various stages of repair will be a key to develop such therapies.

Targeting the Wnt Signaling Pathway to Stimulate Bone Repair

Numerous factors and signaling pathways can potentially be targeted to enhance bone repair. The 2012 ORS meeting highlighted several new advances in understanding the role of the Wnt signaling pathway and molecules that can modulate its functions. McDonald *et al.*[10] reported the phenotype of mice lacking the *Sost* gene, an inhibitor of bone formation expressed by osteocytes and acting by repressing Wnt signaling. *Sost* $^{-/-}$ mice exhibit osteopetrosis and enhanced bone healing marked by increased callus bone mineral content and density, and accelerated endochondral ossification leading to faster union. These results reinforce the therapeutic strategies targeting Wnt antagonists such as treatments with anti-Sclerostin or -Dkk1 antibodies.[11,12] In a rat femoral fracture model, Li *et al.*[13] reported that treatment with Dkk1 antibodies increased bone mineral density and mechanical properties of fractured femurs. Jin *et al.*[14] further showed that Dkk1 antibody acts via β-catenin as it failed to enhance repair in *Prx1CreER;β-catenin*$^{fl/fl}$ mice. Inhibition of Wnt antagonists is also tested to treat bone loss associated with bone aging and osteoporosis. Using microarray, Kitay *et al.*[15] found 20 Wnt pathway genes that are downregulated in the fracture callus of old versus young mice. As fracture healing is also delayed in animal models of aging, these results suggest that inhibition of Wnt antagonists may also benefit elderly patients following bone injury. In addition, Puzas *et al.* found that bone loss following glucocorticoid treatment was mediated by sclerostin upregulation, suggesting that blocking sclerostin may be an effective treatment of steroid-induced osteoporosis.[16] Quirno *et al.* described the role of progressive ankylosis protein (ANK) as a new regulator of Wnt/β-catenin signaling during

fracture repair.[17] ANK-deficient mice showed delayed callus formation, reduced amount of cartilage, bone volume and mineralized areas within the callus compared with wild-type mice. Analyses of bone marrow-derived mesenchymal stem cells in the mutant mice showed that ANK stimulates chondro/osseous precursor cells and stimulates osteoblast differentiation.[18] Using TOPFlash reporter and yeast two-hybrid system, the authors further showed that ANK stimulates Wnt/β-catenin by its interaction with TIPRL, an inhibitor of protein phosphatase 2a (PP2a). PP2a has been shown to negatively regulate Wnt/β-catenin; therefore, ANK may be a new candidate to regulate Wnt signaling and stimulate fracture healing therapeutically.

Cell-based Approaches to Enhance Skeletal Repair

Many laboratories continue to explore cell-based approaches to enhance skeletal repair. Although mesenchymal stem cells (MSCs) have proven their abilities to modulate the immune response and to secrete growth factors influencing bone healing, their contribution as a source of osteochondroprogenitors is still elusive. On the contrary, there is more and more evidence for their poor osteogenic capacities after *in vivo* transplantation. Presentations at workshop #3 entitled 'Current Insights on the Regenerative Potential of the Periosteum: Molecular, Cellular and Endogenous Engineering Approaches' highlighted the importance of local cell recruitment, in particular, from the periosteum, which is a promising source of skeletal progenitors for bone repair.[19–22] Despite this shift in paradigm, many presentations were exploring the recruitment of MSCs at the fracture site after systemic infusion or local transplantation in animal models, assuming MSCs provide skeletal progenitors to support cartilage and/or bone formation. Chin *et al.*[23] investigated the effects of low-intensity pulsed ultrasound (LIPUS) on systemic recruitment of green fluorescence protein (GFP)-labeled MSCs after intracardiac injection. Although fluorescence measurement did not differ in MSC–LIPUS and MSC groups, the authors argued that LIPUS increased MSC homing based on immunohistochemistry. Yet, the identity of GFP-positive cells was not demonstrated. Although improved healing was observed in the MSC–LIPUS group, the mechanism of action remains to be proven. Kodama *et al.*[24] tracked Fe-labeled MSC after transplantation at the fracture site in rats. MSC enhanced bone regeneration but whether they differentiated into osteoblasts is not clear. Several other reports were still under the assumption that a systemic source of osteoblast precursors can be recruited endogenously and exogenously to stimulate repair. Infusion of differentiated osteoblasts appeared promising; however, their long-term integration and therapeutical effects in a model of delayed healing will need to be verified.[25] Conversely, in the report by Bahney *et al.*, direct transplantation of cartilage grafts clearly showed contribution to cartilage and bone in the fracture callus, suggesting a new approach to stimulate bone regeneration.[26] There is no doubt that MSC have therapeutical effects. However, more rigorous methods should be employed to demonstrate the fate of cells after transplantation or in response to treatment X. There are various tools now available to track cells in vivo. For example, Rowe *et al.*[27] illustrated the application of a new Dkk3-GFP reporter mouse in addition to several other GFP reporter mice previously generated in this laboratory to visualize various cell types that participate in fracture repair. Huang *et al.*[28] presented a new multiphoton-based imaging technique to assess at high resolution the interactions among GFP-labeled MSCs with their surrounding matrix and blood vessels in a cranial defect model. Most importantly, these methods must be combined with detailed histological observations to localize the labeled cells and confirm the identity of their progeny.

Conflict of Interest

The author declares no conflict of interest.

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