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REVIEW ARTICLE

Immunological Response to Post-trauma Bone Remodeling

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ABSTRACT

Bone-related immunology (osteoimmunology) is an interdisciplinary translational research field combining orthopaedics and immunology. This review gives an in-depth knowledge in the relationship between the bone trauma and the corresponding changes in host immune system. It also summarizes the most recent developments occuring into this complex field. It has been found that osteoblasts play important role in the maintenance of the hematopoietic stem cell niche and in lymphocyte development as well as the functions of immune cells participating in osteoblast and osteoclast development. Various recent researches are directed to establish the role of cytokines, chemokines, transcription factors and costimulatory molecules, which are shared by both skeletal and immune systems. The understanding of this part of research may open new horizons in the management of bone trauma and that of inflammatory and autoimmune diseases.

Keywords: Traumatic fractures, Bone remodeling, Bone immunology.

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INTRODUCTION

Various common molecular mechanisms as well as various cytokines and signaling transducers participating in the regulatory interplay between immune cells and bone cells, has been identified. Besides the mutual signaling, immune cells and bone cells have common site of origin, i.e. bone marrow. Bones are dynamic, viable, highly organized living tissue and is main constituent of musculoskeletal system.[1]

BONE STRUCTURE AND PHYSIOLOGY

Bone is composed of extracellular matrix (ECM) and cells. ECM can be further subdivided into inorganic and organic part. The organic matrix is mainly constituted of the type I collagen (approximately 95%) and inorganic part predominantly containing calcium and phosphorus, appearing as hydroxyapatite crystals deposited in collagenous matrix. The major cells in bone are osteoclasts (responsible for resorption) and osteoblasts (responsible for bone tissue deposition), converting into osteocytes and bone lining cells. Fully differentiated osteoblasts forming bone lining cells are probably responsible for initiation of remodeling by degrading the matrix. The other fully differentiated osteoblasts are called osteocytes, which acts as mechanosensors (by communicating to each other by means of their dendritic processes) in bone tissue, thus regulating bone mass and bone structure.

Osteoblasts synthesize collagen-rich organic matrix and provided optimal conditions for mineralization by secreting various bone matrix proteins and matrix metalloproteinases (MMP). Mature osteoblasts while depositing matrix and its mineralization, expresses alkaline phosphatase, bone sialoprotein, osteocalcin and osteopontin. As osteoblasts are responsible for matrix, they have prominent golgi apparatus and endoplasmic retinaculum, which are poor in osteocytes.

Osteoclasts are tissue specific and derived from monocytes/macrophage hematopoietic lineage and are the only cells capable of breaking down mineralized bone, including dentine and calcified cartilage. Through integrins, osteoclasts attached tightly to matrix.[2] They create an isolated lacuna, known as Howship's Lacuna with an acidic environment (the ruffled borders of osteoclasts are formed by the fusion of cytoplasmic acidiphilic vacuoles, through which the release of acid-hydrolic acid formed by protons and chloride ions, occurs into the resorption lacuna causing rapid dissolution of hydroxyapatite crystals). The resorption of bone matrix takes place in these resorption lacunas.

BONE REMODELING

In response to varying functional demands, there is continuous adaption by the major and microarchitecture of the bone. This process of adaptation is known as 'remodeling'. It means permanent adaptive micro and macro changes in the architecture of bone. Bone modeling is a result of a balance between bone formation by osteoblasts and bone resorption by osteoclasts. It is a continuous process and at any given time approximately 5 to 25% of bone surface undergoes remodeling. As normal physiological bone remodeling is imperative for the maintenance of bone strength and integrity, any imbalance will either lead to increase or decrease bone mass. By restoring the microdamages, remodeling ensures the mechanical integrity of the skeletal system as well as regulates the release of calcium and phosphorus from the bones into the blood. Remodeling involves four main processes: Activation, resorption, reversal and formation.[3] It is initiated by the activation of the quiescent bone lining cells. The osteoclast precursor cells fuses to form mature osteoclasts. Osteoclasts

initiate dissolution of calcified matrix with specific enzymes. As bone resorption subsides, osteoclasts disappear from resoption pits and mononuclear cells and prepare the ground for new bone formation. Bone remodeling cycle ends with formation of bony canopy of these pits by the osteoblasts, keeping the material dormant until the next cycle.

RESPONSE TO TRAUMATIC FRACTURE

Fracture healing is a unique physiological process resulting in restoration of bone tissues. Growing evidence indicates that the immune cells and secreted factors are crucial for the physiological response following injury.[4-8] Following this inflammatory response, the soluble factors interact with progenitor cells, located in the periosteum, endosteum, bone marrow and surrounding soft tissues.[9] In a traumatic fracture, there is not only injury to bones, but the surrounding soft tissue damage also occurs, resulting into damage to local blood supply. Thus the fractured site and its surrounding develop hypoxia with nutrition deficiency.[10] So, the fracture hematoma is not only characterized by local hypoxia, but also by low pH with high concentration of lactate. Thus, the fracture initiated a local inflammatory response.[11]

Interleukin (IL) 8 upregulation in fracture hematoma (FH) and surrounding hematoma (SH) indicates the same type of inflammatory reaction. The hypoxia-inducible factor (HIF) and its regulation play an important role for the functions of innate and adaptive immune cells. Investigations have proved that it is the initial inflammatory response of fracture, which is crucial for final clinical bone healing. HIF regulates this adaptation by switching the cellular energy metabolism from oxidative phosphorylation toward glycolysis. Hypoxia/HIF reestablishes the normal oxygen supply by promoting angiogenesis via vascular endothelial growth factor (VEGF) and IL-8. Limited and reduced immune response with increasing age may explain the higher chances of delayed/nonunions in these patients.

The exact interplay of immune function and bone regeneration in early (inflammatory) phase is currently unclear.[12-15] Numerous cells are present in the fracture hematoma due to (1) bleeding (peripheral blood leaks through vessel damage into the gap) and (2) broken bone itself (bone marrow flows into the gap). Innate immune cells and CD4+ T cells adapts well to the energy insufficiencies.[16,17]

Progress has been made in understanding the link between adaptive and innate immune system in bone, with emphasis on osteoclasts.[18,19] The function of the adaptive immune system in fracture healing is less understood. Effector cells of the adaptive immune system are lymphocytes. A role of lymphocytes in fracture healing has been suggested but has not been characterized in depth yet. It has already been shown that CD8+ cytotaxic T cells have a counter regulatory role in wound healing because depletion of CD8+ T lymphocytes has a positive influence on wound healing. It has also been established that lack of B lymphocytes can lead to higher bone formation in mice.[18-20]

A study had demonstrated an higher mRNA expression of several genes from FH and SF cells than those in peripheral blood (e.g. osteogenic SSP1, IL-8, CXCR4). It was also seen in the same study that there was decreased expression of the osteoblast differentiation transcription factor RUNX2 in FH and SH from immunologically compromised patients, but there was no difference in SSP1 expression. This fact could indicate that bone regeneration is induced in FH in immunologically compromised patients (but so not in SF). It has been documented that a higher inflammatory response (higher IL-8 and CXCR4 expression) occurs bone regeneration in immunologically restricted patients, but near normal angiogenesis (VEGF) occurs in these patients. It has been postulated that ongoing angiogenesis could be the one of the pathways in bone regeneration. They concluded that there occurs a disturbed (higher and prolonged) inflammatory response with inadequate response to hypoxia (lower expression level of LDHA and PGK1 in fracture hematoma) with normal angiogenesis in immunologically disturbed patients, resulting into prolonged bone healing (delayed union) or nonunion.[13]

In another experiment, the absence of mature lymphocytes was modeled in recombination activation gene 1 knockout mice to look further into the role of lymphocytes to fracture healing. It was postulated that fracture healing would be delayed in the absence of lymphocytes as effector cells of adaptive immune system. The underlying defect in these was loss of recombination activation gene, which encodes for a recombinase that is crucial for the somatic VDJ recombination in the development of B- and T- cell receptors. It was found that lack of lymphocytes led to accelerated bone regeneration in RAG-/- mice. It has also been reported that γ/δ T cells negatively influence fracture healing. It was suggested that probably B cells and not the T cells are predominant during immunoregulation of bone repair.[21]

It has already been discussed that during bone formation, cytokines secreted by lymphocytes have paracrine effects on bone cell differentiation and function. T-helper lymphocyte 1 (THs1) cells promote cellular immunity via secretion of cytokines such as tumor necrosis factor (TNF)-α and LT-β in the callus.[22] It has been conceived that lower levels of TNF-α favors osteogenesis as it acts as a proapoptotic factor on osteoblasts. But it has also been shown that TNF-α signaling impairs fracture healing.[23,24] The positive and negative effects of TNF-α depend upon

its concentration.[21] Activated natural killer cells of innate immune system produce interferon (IFN)-γ in response to bone fracture and maintained its expression for a prolonged period. It causes macrophage activation, leading to tissue destruction.[21]

Lymphopenic mice have displayed earlier and strinkingly higher expression of IL-10, which produced by either myeloid cells or regulatory T cells. IL-10 is a potent inhibitor of secretion of proinflammatory cytokines such as TNF-α, IL-1, IL-6 and T-cell activation. Experiment had shown that during fracture healing, lymphocytes can negatively regulate the amount of IL-10 in callus. IL-10 knockout mice have osteopenic and fragile bones.[25-28] The role of IL-2 has not yet been investigated in fracture healing. But in some experiments in mice, it has been seen that lack of IL-2 enhanced bone formation as IL-2 infusion leads to bone resorption.[21]

The Th2 cytokine IL-4 is considered to exert anti-inflammatory function. It was discovered as a 'B-cell growth factor'[19] and inhibits monocyte production of IL-1, TNF-α and prostaglandin E2 (PGE2). IL-4 inhibits bone resorption and is a chemoattractant for osteoblasts, stimulating proliferation and inhibiting differentiation. During physiologic fracture healing, expression of IL-4 is unregulated.[29] An experiment[30] demonstrated that cells in inflammatory fracture hematoma adapts to hypoxic conditions, which leads to angeogenesis, chemotaxis and osteogenesis (upregulation of VEGF) and IL-6.

Besides numerous other factors, fibroblast growth factor receptor (FGFR) signaling is involved in fracture healing and bone remodeling. FGF23 is a phosphatonin produced by osteoblastic cells, which signals via FGFR1, thereby exerting effects in bone and kidney. A study analyzed if serum FGF23 levels might be an indicator to predict fracture healing and union. It was concluded that FGF23 was involved in bone healing, could be measured by a sensitive assay in peripheral blood and was a promising candidate as an indicator for healing processes prone to reunion *vs* nonunion.[31]

Lymphocytes (T cells) influence bone remodeling through its regulation on osteoclastogevesis. The type and amount of impact of these lymphocytes on osteoclasto-genesis varied from *in vitro* to *in vivo*. The same is true about the type of lymphocyte. The data concerning the effect of CD4 and CD8 lymphocytes on osteoclastogenesis is not consistent. In one study, it was showed that vitamin D3 stimulated osteoclast-like cell formation was increased in presence of lymphocyte depletion. It was postulated that it was due to more PGE2 production, leading to upregulated RANKL and downregulated OPG expression.[32-35]

There is little detailed information on the cytokines production pattern of osteoblasts. IL-6 is produced by the stromal cells/osteoblasts.[36] A number of growth factors and hormones are known to promote proliferation and differentiation of osteoblasts, such as TGF-β (is also assumed to suppress osteoblast differentiation),[37] parathyroid hormone, its locally produced homologue parathyroid hormone-related peptide (PTHrP), low density lipoprotein receptor-related protein-5 (LRP-5),[38] and osteopontin.[39] In recent research, much attention has been attributed toward bone morphogenic proteins (BMPs). In pigs, BMP-6 and BMP-7 have been shown to increase osteogenic differentiation *in vitro* and BMP-4, besides IGF-1 and TGF- β, was found to be expressed during distraction osteogenesis in a pig model.[40] Production of IL-6 and RANKL by osteoblasts is promoted by PTH and TNF-α. Whereas PTH induces only a rapid, but transient elevation of both cytokines, TNF-α leads to a biphasic increase of these cytokines, thus is indicating the potential role of TNF-α in pathologic conditions.

CONCLUSION

It is likely that cytokine effects on fracture healing can be divided into noxious and beneficial. In case of injury, this might reflect the inherent physiologic compromise producing defense mechanisms and reparative potential at the same time. New immunomodulatory intervention strategies to improve fracture healing could specifically target lymphocyte subsets or cytokines to eliminate detrimental action and to preserve necessary leverage. The clinical implications of these observations are unclear, but we speculate that facilitating these effects therapeutically may enhance fracture healing.

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