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Review Article

New perspectives on traumatic bone infections

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ABSTRACT

In this paper, we review the results of previous studies and summarize the effects of various factors on the regulation of bone metabolism in traumatic bone infections. Infection-related bone destruction incorporates pathogens and iatrogenic factors in the process of bone resorption dominated by the skeletal and immune systems. The development of bone immunology has established a bridge of communication between the skeletal system and the immune system. Exploring the effects of pathogens, skeletal systems, immune systems, and antibacterials on bone repair in infectious conditions can help improve the treatment of these diseases.

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Introduction

Bone infection leads to progressive bone destruction, aberrant bone formation and systemic inflammatory response. Among the bacteria species isolated from bone infections, *Staphylococcus aureus* is the most prevalent bacterial subtype, especially for bone infections due to trauma. Although our understanding towards the pathophysiology and treatment of bone infections has progressed significantly, it remains a clinical conundrum for orthopedic surgeons, especially cases caused by open fracture trauma.[1] The skeletal system relies on osteoblasts, osteoclasts, and other functional cells in the microenvironment to maintain a dynamic balance. With age and decreased hormone levels, bone absorption gradually dominates, eventually leading to systemic bone destruction. Both severe traumatic stress and post-infectious inflammatory state accelerate the process of bone destruction. Effective anti-osteoporosis treatment promotes systemic bone deposition and reduces the incidence of age-related and postmenopausal-related fractures. At the same time, conditions involving inflammation, such as rheumatoid arthritis, septic arthritis, and traumatic osteomyelitis, also affect the normal functioning of the skeletal system, most of which are accompanied by varying degrees of bone destruction.[2] When infections are around the bone, the local tissue destruction caused by the reaction poses a massive challenge to clinical treatment. At present, the treatment of such diseases is gradually standardized, but further understanding of the pathological processes of different types of bone destruction is equally crucial for the introduction of effective prevention and treatment.[3]

The immune system and the skeletal system are in a skeletal microenvironment, and the two systems are closely related and cooperate with each other. Immune cells and their metabolites are involved in the regulation of bone deposition and bone strength by affecting the activity of osteoblasts, osteoclasts, and bone cells (Table 1). Studies of the association between the two systems began with the discovery of the bone resorption promoting factor IL-1β 40 years ago.[4] The development of the subsequent osteoclast important differentiation factor, receptor activator of nuclear factor-κB ligand (RANKL), further unveiled the mystery of osteoimmunology.[5] Many studies revealed the effects of numerous cytokines such as TNF-α, IL-1β, and IL-17 on inflammatory bone diseases. At the same time, specific antagonists of these cytokines have also been developed. Osteoimmunology studies also provide evidence for the interaction between estrogen deficiency and immune response activation in the pathogenesis of postmenopausal osteoporosis.[6] All of the above evidence may offer new perspectives for the prevention and treatment of traumatic bone infections.

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Table 1
Part of immune cells involved in bone metabolism.

Cell type	Cytokine	Bone metabolism target	References
Th1 cell	IFN-γ, IL-2, TNF-α, and GM-CSF	Inhibit osteoclast formation (IFN-γ degrades TRAF6)	36
Th2 cell	IL-4, IL-5, IL-10, and IL-13	Inhibit osteoclast formation (IL-4, IL-5, and IL-13). Maintain anabolic activity of osteoblasts (promote the synthesis of PTH).	37,38
Th9 cell	IL-9	Promote osteoclastogenesis (synergistically induces differentiation of CD4 + T cells into Th17 cells with TGF-β).	39
Th17 cell	IL-17A, IL-17F, IL-21, and IL-22	Promote osteoclastogenesis (IL-17 promotes effector cell expression of RANKL). Inhibit osteoblast formation (IL-17 inhibits BMP-2 induced osteoblast differentiation). Promote bone resorption (IL-22 promotes the absorption activity of osteoclasts).	40–42
Treg cell	CTLA- 4, IL-10	Inhibit osteoclast formation (CTLA-4 inhibits the production of RANKL and M-CSF). Inhibit osteoclast formation (IL-10 selectively inhibits calcium signaling downstream of RANK by inhibiting TREM-2 transcription).	43
NKT cell	IL-15, TNF-α, and IFN-γ	Recruiting monocytes (Upregulation of chemokine receptor and adhesion molecule expression). Promote osteoclastogenesis (IL-15 and RANKL synergistically induce osteoclastogenesis). Bidirectional regulation of osteoclast differentiation (TNF-α, IFN-γ).	44,45
γδT cell	IL-6, IFN-γ	Activate γδT cells to affect the regulation of osteoclastogenesis (IL-6 produces a promoting effect. IFN-γ production inhibition).	8

TRAF6: TNF receptor-associated factor 6, PTH: parathyroid hormone, RANKL: receptor activator of nuclear factor-κB ligand, CTLA- 4: CD80/cytotoxic T-lymphocyte associated protein 4, NKT: natural killer T.

Traumatic bone infections

The main factors causing pathological changes in traumatic bone infections include stress response, activated immune response, pathogenic factor, and disturbed bone metabolism. The staphylococcal microbiota that accumulates in the wound is also involved in the process of bone metabolism. Pathogens are agonists of immune response and have a direct effect on bone metabolism. On one side, an immune response caused by traumatic bone infections leads to severe bone destruction. On the other, bacteria cause bone destruction by directly killing osteoblasts, and the immune response elicited by bacteria is also crucial for osteoclast differentiation and bone degradation. The most representative of these pathogens is *Staphylococcus aureus*.[7] During infection, these bacteria can survive in the bone microenvironment by forming biofilms and triggering a severe immune response. At the same time, host cells secrete many cytokines, such as TNF-α, IL-1β, IL-6, IFN-γ, chemokine (C−C motif) ligand 3 (CCL-3), (C-X-C motif) ligand 1 (CXCL1), and chemokine (C-X-C motif) ligand 2 (CXCL2).[8] The hemolytic toxins produced by *S. aureus*, bacterial lipoproteins, and Panton-Valentine leucocidin (PVL) can stimulate immune cells to secrete IL-1β. In this process, even though the individual products of specific immune responses result in a negative regulation of bone resorption, the overall effect is still severe bone destruction.[9] *S. aureus* protein A can promote the secretion of NF-κB by the binding of TNF R1 on the surface of osteoblasts, resulting in the secretion of IL-6. The peptidoglycan component of bacteria promotes the secretion of RANKL and reduces the production of osteoprotegerin (OPG) by activating the Toll-Like receptor-2 pathway.[10] Trouillet-Assant et al.[11] reported that bone marrow-derived osteoclast precursors were infected by living *S. aureus* to induce differentiation into active macrophages accompanied by secretion of many pro-inflammatory cytokines. On the one hand, these cytokines enhance the bone resorption function of uninfected mature osteoclasts. On the other hand, these cytokines promote the differentiation of uninfected osteoclasts. In addition to mediating bone destruction by immune cells, *S. aureus* also directly affects osteoblasts. Josse et al.[12] reported that *S. aureus* could reduce the osteogenic activity and proliferative activity of osteoblasts by inducing apoptosis-dependent manner.

Dentin destruction is another frequently encountered condition of infection-related bone destruction, which presents with many features similar to osteomyelitis. Rhodobacter sphaeroides is one of its major pathogens.[13] IL-17 is a type of cytokine that is locally and highly expressed in periodontitis and plays an essential role in inflammatory response and bone destruction. Rhodobacter sphaeroides also secretes a cysteine proteolytic enzyme called gingival protease, which does not directly reduce osteoclast formation but reduces the production of osteoclast regulatory factors, such as OPG.[14] Almost all types of Streptococcus pyogenes produce streptolysin O (SLO). Our previous study found that SLO reduced phosphorylation of p65 and IκBα in the signaling pathway of osteoclast differentiation, thereby inhibiting the expression of c-Fos and nuclear factor of activated T cells 1 (NFATc1) and downregulating the expression of osteoclast marker genes. SLO also induces apoptosis in mature osteoclasts, suggesting that SLO blocks osteoclast activation during S. pyogenes infection.[15]

Osteoclasts are essential effectors for infection-related bone destruction

As the function of RANKL is gradually revealed, the role of osteoclasts in bone metabolism becomes clearer. This functional cell is involved in the process of infection-related bone destruction. Mature osteoclasts degrade bone matrix proteins by secreting proteolytic enzymes and release the inorganic components of decalcified bone by releasing hydrochloric acid. Under physiological conditions, the binding of RANK superfamily member RANKL to its ligand RANK regulates the formation of osteoclasts.[5] High doses of RANKL promote osteoclast differentiation, while low doses of RANKL reduce osteoclast differentiation.[16] RANKL and RANK signaling requires the recruitment of TNF receptor-associated factor 6 (TRAF6), which in turn activates the downstream mitogen-activated protein kinase (MAPK) and NF-κB pathways, ultimately leading to the expression of the critical transcription factor NFATc1 in osteoclast formation.[17,18] High expression of RANKL promotes osteoclast differentiation, a process that can be inhibited by OPG, which is a high-affinity bait ligand for RANK that competes with RANKL for binding to RANK for negative regulation of osteoclastogenesis. Deletion of OPG can lead to systemic osteolytic lesions.[19] The binding of macrophage colony-stimulating factor (M-CSF) to its ligand colony-stimulating factor-1 receptor also plays an essential role in the differentiation of osteoclasts.[20]

The extent of bone resorption depends on the number of osteoclasts and bone resorption activity. The formation of osteoclasts

under physiological conditions is controlled by the expression levels of RANKL, M-CSF, and OPG, which are also regulated by many inflammatory factors in infection-related bone diseases. RANKL promotes the differentiation of osteoclast precursor cells into osteoclasts, and M-CSF promotes the proliferation of precursor cells and inhibits apoptosis. In addition to the stage of cell differentiation, bone absorption is also a complicated process. First, the osteoclasts are oriented to bone migration by integrin-like proteins on the cell surface, such as αVβ3, thereby forming a closed cavity.[21] High concentrations of RANKL and M-CSF in infection-related bone destruction can both increase the number of osteoclasts and promote the phagocytosis of mature osteoclasts. The relationship between numerous cytokines and the interaction of these two osteoclast key factors is essential for a better understanding of bone destruction.[22]

Immunomodulation of traumatic bone infections

Immunomodulation is essential for the development of traumatic bone infections. The process of post-traumatic tissue repair begins with the activation of coagulation, stimulating the release of various growth factors, such as vascular endothelial growth factors, platelet derived growth factors, and fibroblast growth factors. Meanwhile, inflammatory cells (macrophages, neutrophils, and T-lymphocytes) accomplish phagocytosis and the removal of bacteria, cellular debris, and damaged tissue.[23] Macrophages are both secretory cells of many inflammatory factors and precursor cells for osteoclast differentiation.[24] The process by which macrophages differentiate into osteoclasts is influenced by many factors, such as the relative proportions of M1 and M2 cells.[25] M1 and M2 macrophages play an essential role in the immune response. IL-4 and IL-13 secreted by Th2 lymphocytes and eosinophils are both critical to the differentiation of M2 macrophages, and they also inhibit osteoclast formation.[16] TNF-α is a factor that promotes the formation of osteoclasts, and it also promotes the accumulation of IL-13-producing eosinophils to the focal joints. Although researchers have suggested that osteoclasts are not derived from activated M2 macrophages, osteoclasts isolated from mouse arthritis specimens possess the characteristics of M1 and M2 macrophages.[26] Thus, the differentiation of osteoclasts is highly dependent on the microenvironment in which the cells are located.

The regulation of the immune system on bone metabolism can be manifested as a dual role in reducing osteoclast formation and anti-bone resorption. For example, CD4+ FoxP3+ T cells are both effective suppressor cells for osteoclast differentiation, and they also exhibit certain anti-inflammatory effects. These cells directly inhibit the binding between CD80/cytotoxic T-lymphocyte associated protein 4 (CTLA4) required for osteoclast formation.[27] Another type of CD8-positive T cells reduces osteoclast formation by direct contact. These cells not only inhibit osteoclast differentiation in vitro but also inhibit bone destruction in RANKL-injected and ovariectomized mice.[28] Th1 cells are thought to play a role in promoting osteoclast formation because they also secrete RANKL.[29] At the same time, Th1 cells can also secrete IFN-γ, which inhibits this process.[30] CD4+IFN-γ+ T cells promote osteoclast differentiation by secreting RANKL, which is detected in large numbers in patients with rheumatoid arthritis.[31] Therefore, IFN-γ produced by Th1 cells results in a direct and indirect inhibition of osteoclast formation.

As important immune cells, B cells play a role in not only inducing antigen-dependent T cells but also producing antibodies under healthy and infected conditions. Various effects mediated by B cells also include the secretion of cytokines and chemokines that mediate cell differentiation and inflammation, and are involved in the regulation of the RANK/RANKL/OPG system. Therefore, B lymphocytes play a key role in bone homeostasis, osteoclast formation, and osteogenesis.[32] Although research in 1998 demonstrated that B cells secrete anti-osteoclast factor OPG, historically, scholars believe that the main source of OPG is osteoblasts. Subsequent research has challenged this view. Researchers have found that B cells are the main source of OPG in mouse bone marrow (BM) under physiological conditions, accounting for 64% of total BM OPG production.[33] Under infection, B cells serve as an important source of the osteoclastogenesis factor RANKL.[34] In contrast, some scholars believe that B cells can inhibit the formation of osteoclasts by producing TGF-β and IFN-γ.[35] Overall, further research is needed to determine how B-cell-mediated immune responses are involved in the activation of osteoclasts in infection-related bone destruction.

Effect of antibiotics on the regulation of bone metabolism

Antibiotic is a component of the treatment of traumatic bone infections, and it also has an important impact on bone metabolism. Antibiotic-loaded bone cement is widely used in the repair of traumatic bone defects. Gentamicin is an early antibiotic used in bone cement mixed preparations. As bone cement is used more frequently, researchers have used mouse skulls to find that the use of gentamicin may result in inhibitory effect on bone tissue similar to its nephrotoxicity.[46] In another study using the C2C12 cell line as an osteoblast lineage, the researchers reduced the cell viability and alkaline phosphatase activity of cultured cells by topical application of high concentrations of gentamicin. Researchers also confirmed that gentamicin might be harmful to bone healing and repair in vivo.[47] Researchers applied different high-concentration antibiotics in the culture of primary human osteoblasts, and they measured the concentration of lactic acid in the cell culture supernatant as an indicator of glycolysis. The researchers observed that clindamycin, fluoroquinolone, linezolid, chloramphenicol, rifampicin, and tetracycline might produce cytotoxicity and a cytostatic effect on primary human osteoblasts by mitochondrial energy supply.[48] Vancomycin is a glycopeptide drug used in severe infections caused by drug-resistant strains. Researchers selected eight concentrations of 21 different types of antibiotics to stimulate osteoblasts, and they measured osteoblast deoxyribonucleic acid content and alkaline phosphatase activity to assess cell number and osteogenic activity. The results confirmed that amikacin, tobramycin, and vancomycin resulted in the least cytotoxicity, and high concentrations of drugs significantly affected cell proliferation and ALP activity. The data provide effective theoretical support for the local application of vancomycin in bone infections.[49]

The post-puberty phase of skeletal development is a critical period of plasticity, supporting approximately 40% of the peak bone mass. Hathaway-Schrader et al.[50] reported that gut microbiota contribute to bone health. The team treated mice with a mixture of three antibiotics and found that antibiotic treatment led to changes in the intestinal microbiota of mice, which ultimately led to specific changes in the bacteria. As antibiotics destroy the intestinal microbiota, the researchers examined the integrity of the skeletal system and found that antibiotic-induced microflora changes exhibited little effect on cortical bone but caused high metabolic changes in trabecular bone. The results showed that after antibiotic

treatment, osteoblast proliferation and differentiation did not change, while the number, size, and activity of osteoclasts increased. Further research deepened into the specific immune mechanisms of the bone marrow environment, demonstrating the effects of antibiotic treatment on bone marrow cells. By detecting immune cell populations in the bone marrow, the researchers found a significant increase in animal bone marrow-derived suppressor cells (MDSCs) in the antibiotic-treated group. MDSCs can regulate innate and adaptive immune responses in a variety of diseases.

Despite the direct effect of antibiotic toxicity on the bone cell line in bone infections, antibiotics should be absorbed mostly by the reduction of bacteria. Optimizing the route of administration and selecting sensitive antibiotics as soon as possible are critical in reducing the effects of its toxicity on cells.

Prospects for treatment

In the past, many patients with traumatic bone infections eventually lost mobility due to severe bone destruction. Surgery is the only option for them. As the pathogenesis of traumatic-related inflammatory bone destruction is further revealed, especially in understanding the interrelationship between the immune system and the skeletal system, important advances have been made in the treatment of such diseases. For example, the use of the RANKL inhibitor Denosumab is based on the inhibition of excessive differentiation of osteoclasts in inflammatory bone destruction.[51] However, this treatment exhibits no anti-inflammatory effect, so it is also necessary to combine anti-inflammatory therapy. Several inhibitors of inflammatory factors are currently used to treat inflammatory diseases, including targeted inhibition of IL-1, IL-6R, IL-17, IL-12/23, and TNF-α. The TNF-α inhibitor exhibits both anti-inflammatory and inhibitory functions of osteoclast formation. The IL-6R inhibitor, IL-12/23, and IL-17 inhibitor used in patients with rheumatoid arthritis all exhibit the above dual effects. IL-1 antagonists directly inhibit the differentiation and functional activation of osteoclasts while reducing the inflammatory response.[52] IL-1 inhibitors are mainly used in diseases where IL-1β is the main causative factor, such as gout.[53] Another small molecule inhibitor of NLRP3 inflammatory corpuscle has also been used in many mouse disease models, demonstrating that inflammatory corpuscles are also an effective target for such diseases, but there are no clinical reports of such drugs.[54] Drugs that regulate the acquired immune response, especially those that regulate T cell activity, also exhibit a function of effectively regulating bone resorption. For example, CTLA4 expressed in patients with rheumatoid arthritis inhibits osteoclast differentiation by inhibiting T cell activation and binding to osteoclast precursor cells.[55]

In general, the immune system is closely involved in the disease process of the skeletal system, and complex communication between the two systems needs to be further elucidated. The study of immune pathways regulating bone metabolism has been conducted to help us better understand these diseases. The development of basic research promoted the advancement of therapeutics, such as targeted therapy of cytokines in pathogenic factors, which can selectively resolve the cause, reduce the inflammation, delay the bone resorption, and thus treat the infection-related bone destruction.

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Ethical Statement

Not applicably.

Declaration of Competing Interest

The authors declare that there are no conflicts of interest.

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