The Role of Immune Microenvironment in Maxillofacial Bone Homeostasis

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Maxillofacial bone defects are common medical problems caused by congenital defects, necrosis, trauma, tumor, inflammation, and fractures non-union. Maxillofacial bone defects often need bone graft, which has many difficulties, such as limited autogenous bone supply and donor site morbidity. Bone tissue engineering is a promising strategy to overcome the above-mentioned problems. Osteoimmunology is the inter-discipline that focuses on the relationship between the skeletal and immune systems. The immune microenvironment plays a crucial role in bone healing, tissue repair and regeneration in maxillofacial region. Recent studies have revealed the vital role of immune microenvironment and bone homeostasis. In this study, we analyzed the complex interaction between immune microenvironment and bone regeneration process in oral and maxillofacial region, which will be important to improve the clinical outcome of the bone injury treatment.

Keywords: immune microenvironment, bone homeostasis, osteoimmunology, cytokines, immune cells (ICs)

Bone resorption and bone formation are largely regulated by a variety of immune responses under normal and pathogenic conditions. Full understanding of the principles in bone homeostasis is vital to treat patients with traumatic injuries, osteonecrosis, arthritis, bone infection, osteoporosis, metabolic bone disease, tumors (1). Maxillofacial bone is a dynamic tissue and maintains a constant balance between bone loss and subsequent repair with participation of the immune system to a large extent (2). Over the years, maxillofacial bone defects are difficult and challenging problems for maxillofacial surgeons resulting from tumor, trauma, congenital defects, reconstructive surgery, non-union of fractures, infection, or periodontal disease. Repair, reconstruction together with regeneration in bone defects remain a challenge in the oral and maxillofacial region (3). Osteo-immunology has opened the field that explored the complex cellular and molecular networks involved in oral maxillofacial osteolytic diseases, explored the interaction between metabolism of bone and immune response and provided background for the study of chronic inflammatory disease associated with bone loss (4, 5). Periodontal health depends on the local balance among immune cells, cytokines and mediators (6). Simultaneously, bone homeostasis is closely related to immune cells and immune derived cytokines. The challenge is to require this homeostatic equilibrium of the oral microbiome, the moderate inflammation and the adaptive alveolar bone remodeling. The main purpose of this review is to explain the effect of osteo-immunology on maxillofacial bone.

THE ROLE OF IMMUNE CELLS IN BONE REGENERATION

Bone homeostasis is a highly coordinated process responsible for bone formation, bone repair and bone remodeling. In addition to the traditional bone cells, immune cells containing neutrophils, B cells, macrophages and T cells, were also implicated in remodeling of bone (7). In osteolytic inflammatory diseases, such as osteoarthritis of the temporomandibular joint, periodontal disease, apical periodontitis and maxillofacial bone sarcomas, inflammation leads to tissue destruction, especially bone loss by the continuous release of osteoclastogenic mediators. Mandible and maxilla are the only bones direct exposure to a microbial contaminated environment, such as periodontal disease or during and after surgical procedures, containing tooth extraction, some resection of jawbone tumors and so on (7). The acute innate immune response is crucial in the early stage of bone healing after injury, which depends on immune cells and cytokines (8, 9). Immune cells are essential in bone repair by sensing the extracellular signals, eventually drive bone remodeling by regulating osteogenesis or osteoclastogenesis (10). Therefore, interaction between local stem cells and immune cells in the oral microenvironment may modify the regenerative process (11) (see **Figure 1**).
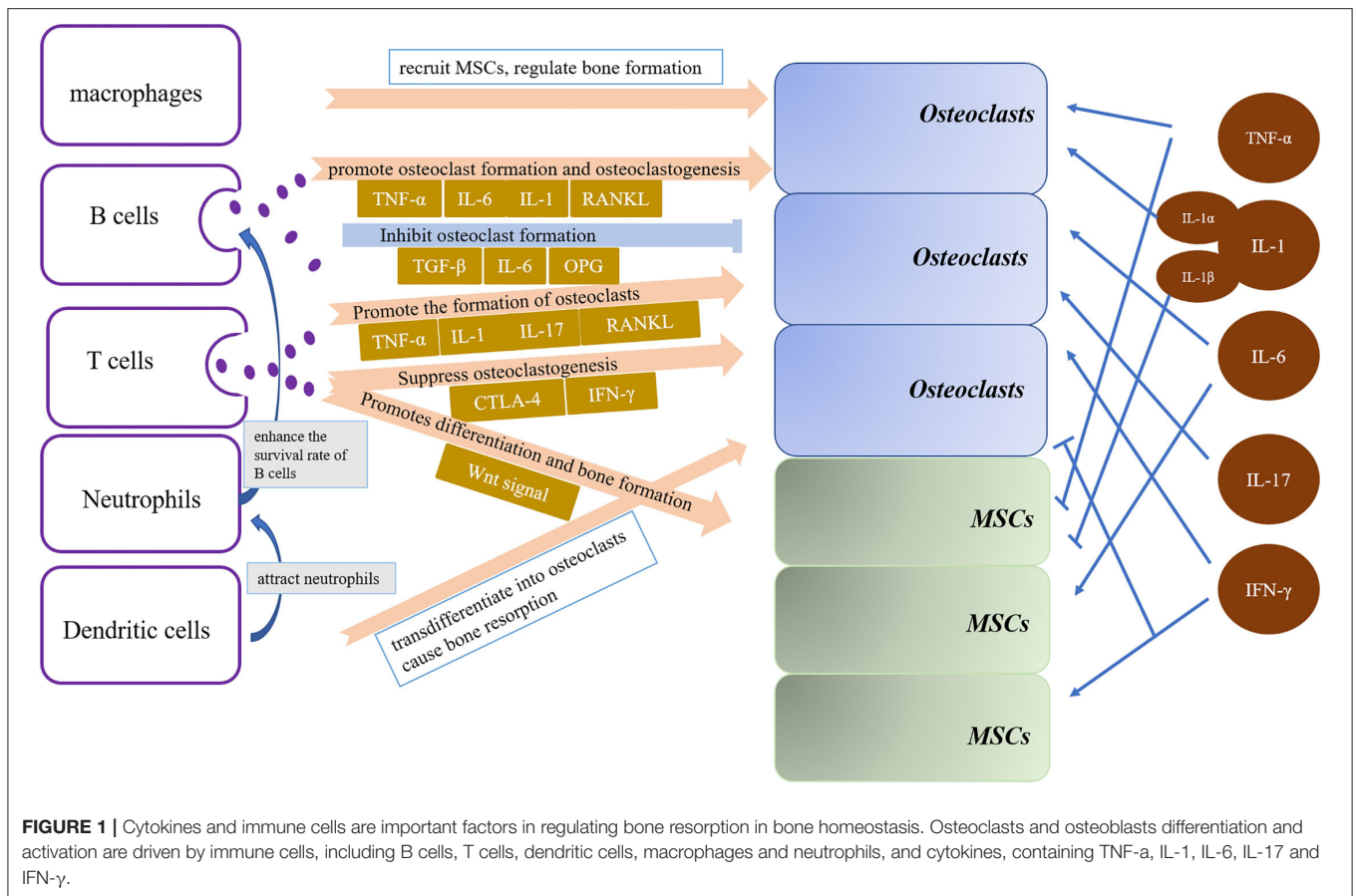
Neutrophils

Neutrophils, which are a type of polymorphonuclear leukocytes, are the most abundant immune cells in human peripheral blood (12). Neutrophils are universally acknowledged as one of the major participants in acute inflammation (13, 14). Neutrophils often act as the front line of defense to be recruited to an inflammatory site to fight off pathogens by eliminating pathogens, cell debris as well as microorganisms (15, 16). Neutrophils play a critical role in the acute inflammatory response and wound healing, especially at the beginning of hemostasis and inflammation (12). Neutrophils are also all importantly influential in chronic inflammatory or aging-related diseases, including periodontitis, diabetes and rheumatoid arthritis (17). Furthermore, periodontitis is related to increased risk of certain systemic diseases, such as atherosclerosis and rheumatoid arthritis (18). Neutrophils are involved in periodontal inflammatory responses. Neutrophils are accumulated in periodontal tissues and also enriched in the fluid of gingival crevice (19, 20). Neutrophils are increased in the process of inflammation and are vital for periodontal tissue homeostasis (21, 22). Neutrophils can secrete cytokines that enhance the survival rate of B cells and plasma cells, which is associated with periodontitis and has a causal relationship with periodontal bone loss in mice (23). Neutrophils show heterotypic adhesion to osteoblasts and regulate the function of osteoblasts in osteoimmunological regulation of periodontal diseases (24, 25). Neutrophils play an important part in the pathogenesis of bisphosphonate-associated osteonecrosis of the jaws and impaired normal wound healing (26).

Macrophage

Macrophages are cells of innate immunity that are present in nearly all tissues, where they make a substantial contribution to development, homeostasis and regeneration (27, 28). In fracture healing, macrophages migrated into the fracture area and had an impact on the long term outcome of bone repair (29). Macrophages can not only remove the temporary fibrin matrix, necrotic cells and damaged tissues *via* phagocytosis at the fracture site but also recruit vascular progenitor cells and mesenchymal stem cells (MSCs) from the bone marrow, periosteum and circulation (30). Therefore, bone repair requires a long-time regenerative response to achieve anatomical and functional recovery of bone. Macrophages are essential for the initiation of bone repair and also participate in the regulation of bone regeneration during normal bone homeostasis (31). Bone macrophages *in vivo* are close to osteoblasts, can regulate bone formation, support fracture healing and play a variety of roles in the potential role of osteocyte proliferation in bone biology and the regulation of bone metastasis (27). Macrophages are responsible for the homeostasis and functions of the alveolar bone (32). Macrophages are only a few in periodontal tissues, but they participate in the pathogenesis of periodontitis by initiating or eliminating inflammation, mediating alveolar bone resorption and localization (33–35). Therefore, the monocyte/macrophage population in alveolar bone participates in the regulation of MSCs and bone homeostasis, which promotes osteogenic differentiation and inhibits adipogenic differentiation of MSCs (36). Together, these results suggest that macrophages function in regulating physiological bone formation and homeostasis.

Dendritic Cells

Dendritic cells are a special type of antigen-presenting cells that can capture, process and present antigens to lymphocytes, so as to initiate and regulate the adaptive immune response (37, 38). Dendritic cells activate a protective antibody response, thereby reduce bone loss (39). Immature dendritic cells may directly participate in the regulation of osteoclasts and lead to bone decline in histiocytosis, rheumatoid arthritis or periodontitis (40, 41). Chemokines secreted by dendritic cells can attract monocytes and neutrophils to sites of inflammation, so as to enhance inflammation and stimulate the expression of osteoclastogenetic factors (42). Dendritic cells can transdifferentiate into osteoclasts and furthermore cause bone resorption in patients with periodontal inflammation (43, 44). Dendritic cells can enhance periodontal bone loss by upregulating Th17 or Th1 response (45). Results in dendritic cells-deficient mice confirmed that dendritic cells deficiency could contribute to bone necrosis after tooth extraction (46). Dendritic cells are essential in initiation and regulation of immune responses in the clinical case of oral cavity (46, 47). Previous studies reported insufficient innate immune response and colonization of oral bacterial communities in bisphosphonate-related osteonecrosis of the jaw (48). Dendritic cells also play a major role in the oral mucosal barrier immunity of the oral cavity and the gut mucosa (49).

FIGURE 1 | Cytokines and immune cells are important factors in regulating bone resorption in bone homeostasis. Osteoclasts and osteoblasts differentiation and activation are driven by immune cells, including B cells, T cells, dendritic cells, macrophages and neutrophils, and cytokines, containing TNF-a, IL-1, IL-6, IL-17 and IFN-γ.
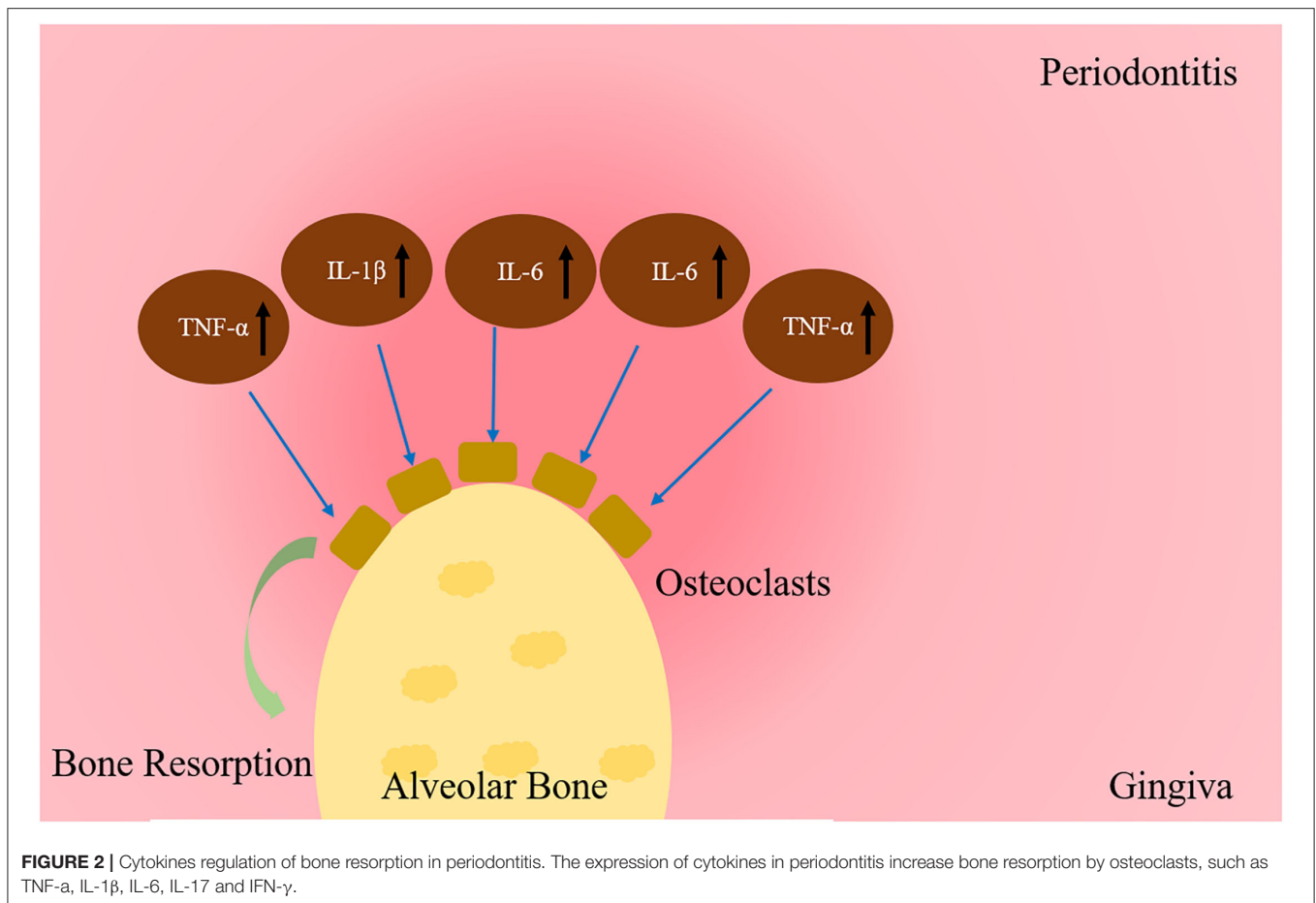
T Lymphocytes (T Cells)

T lymphocytes participate in host defense and control the development of immune-mediated inflammatory disease (50). According to the original concept of bone immunology, T cells are critically involved in the mediation of inflammatory repose guided bone loss. Activated T cells indirectly or directly regulate bone health and bone remodeling by secreting various cytokines and factors (50, 51). For example, activated T cells are primary sources of receptor activator of TNF-α and nuclear factor-κB ligand (RANKL) responsible for bone destruction observed under pathological and inflammatory conditions (52). Activated CD8$^+$ and CD4$^+$T cells secrete interleukin-1 (IL-1), tumor necrosis factor (TNF-α), receptor activator of nuclear factor-κ B ligand (RANKL), IL-6, and IL-17 to promote the formation of osteoclast (53). FoxP3$^+$ CD8$^+$ T-lymphocytes produce CTLA-4 and interferon-γ (IFN-γ) to suppress osteoclastogenesis while produce RANKL to enhance osteoclastogenesis (54). However, CD8$^+$ T cells can produce Wnt10b to mediates activation of Wnt signal while γδ T cells secrete IL-17A to accelerate bone regeneration (55). Wnt signaling plays crucial roles in postnatal bone formation. Wnt signaling can promote the commitment of mesenchymal stem cells (MSCs) to osteoblastic lineage and promotes differentiation and bone formation at the critical steps of osteoblast differentiation (56). T lymphocytes and B lymphocytes can result in the production of RANKL, which

causes osteoclasts to induce obvious alveolar bone resorption, even tooth loss (57).

B Lymphocytes (B Cells)

B cells are an important branch of the adaptive immune system and can also present antigens and secrete antibodies and cytokines (58). Recent studies have disclosed a regulatory effect of B cells, indicating that B cells affect osteoclasts by producing cytokines (59, 60). It had been reported that B cells can produce TGF-β, IL-6, OPG to inhibit osteoclast formation (61). It is reported that several B cells in healthy gingiva may have an important role to play in preventing bone loss caused by inflammation of periodontium (62). However, B cells can express TNF-α, IL-6, and RANKL to promote osteoclast formation and osteoclastogenesis (60). B cells have been closely related to periodontal homeostasis and disease (57). B cells express RANKL for alveolar bone homeostasis in homogeneous gingival tissue during periodontal disease (63, 64). B cells also play a vital role in attachment loss and alveolar bone resorption in periodontitis in mice, which may be due to activation of B cells and the expression of RANKL in the gingiva (65). Furthermore, B cells deficiency reduces alveolar bone resorption during periodontitis (66). Furthermore, memory B lymphocytes can lead to bone damage in rheumatoid arthritis (67). And

FIGURE 2 | Cytokines regulation of bone resorption in periodontitis. The expression of cytokines in periodontitis increase bone resorption by osteoclasts, such as TNF-a, IL-1β, IL-6, IL-17 and IFN-γ.

B lymphocytes in periodontitis may result in chronic systemic inflammation (68).

THE ROLE OF IMMUNE CYTOKINES IN BONE REGENERATION

Cytokines are intercellular regulators at systemic and local level. Cytokines are derived from immunocompetent cells such as monocytes and T lymphocytes. Chemokines manage the location and migration of immune cells and are essential in the function of the innate immune system. Chemokines can also affect bone marrow to release innate immune cells throughout development, homeostasis, and inflammation (69). Periodontal tissue such as epithelial and endothelial cells as well as fibroblasts are also involved in cytokine formation during inflammatory responses (70, 71). Besides, various cytokines are directly related to the occurrence of osteoporosis in animal models and patients (72). Several chemokines produced by osteophytic tumor cells can promote osteoclasts-mediated bone resorption and promote the osteoclast precursors recruitment and osteoclast precursors differentiation (73).

Tumor Necrosis Factor Alpha (TNF-α)

TNF-α mainly secreted by monocytes, can stimulate the activity of mature osteoclasts, and attract other monocytes (74). Furthermore, TNF-α frequently appeared in the tumor microenvironment and is mainly derived from tumor cells and tumor-associated macrophages (75). Once cancer cells are detained in bone, the bone stores diverse growth factors and cytokines and thus provides an extremely fertile environment for cell growth (76). Invasive tumor cells secrete TNF-α, interleukins and chemokines and change the bone microenvironment, which directly induce osteoclasts and/or promote RANKL expression in osteoblasts and stromal stem cells (77). It was firstly described that TNF-α inhibited bone formation in neonatal rat calvarial organ cultures in 1987 (78). Recently, TNF-α plays a critical role in the pathogenesis of inflammatory bone loss by stimulating osteoclasts bone resorption and inhibiting osteoblast bone formation (79, 80). Further studies showed the inhibitory effects of TNF-α in recruitment of osteoblast progenitors, genes expression produced by mature osteoblasts, and the active influence in osteoblast apoptosis *via* nuclear factor kB (NF-κB) pathway (81). In many chronic and inflammatory disease, TNF-α plays a negative role in regulating bone homeostasis (79, 82, 83). TNF-α was obviously upregulated in the process of periodontitis (84), which is actively involved in the destruction

of periodontal tissues by regulating the activities of leukocytes, osteoclasts (85). Furthermore, the expression levels of TNF-α was closely associated with the severity of periodontitis (86) (see **Figure 2**).

Interleukin-1 (IL-1)

IL-1 plays vital role in immune and inflammatory responses (87). The systemic IL-1 has important roles in regulation of basic metabolic rate, iron metabolism and bone remodeling (88). IL-1 stimulates bone resorption by promoting osteoclast activation (89, 90) and mediates osteoclatogenic effects of TNF-α by up-regulating the expression of RANKL (91). IL-1 also has a prominent role in the pathogenesis of periodontitis (85). The IL-1 family contains two main members, IL-1α and IL-1β. IL-1α promoted osteoclast differentiation by stimulating prostaglandin E2 (92). IL-1α can also exert its biological effects on the alveolar bone modeling process of tooth eruption by enhancing RANKL and TNF-α expressions (93). Previous studies showed that IL-1α is an inducer, which could up-regulate the expression of matrix metalloproteinases (MMPs), such as MMP 13, 9, 7, 1, and 3 in the process of infection or the formation of endodontic and periodontal osteolytic lesions (94–96). IL-1β was detected to inhibit osteogenetic and adipogenetic differentiation of MSCs (97). As one of the important pro-inflammatory mediators, IL-1β is increased in the early stage of fracture healing (98). IL-1β affects alveolar bone resorption in ligature-induced chronic periodontitis by enhancing osteoclastic differentiation (99) (see **Figure 2**).

Interleukin-6 (IL-6)

IL-6 has been reported to influence osteoclastic differentiation and bone resorption (100, 101). IL-6 exerts a significant impact on immune responses and certain oncological conditions (102). There are studies suggesting that IL-6 is a potential biomarker for oral squamous cell carcinoma in oral cavity and oropharynx (103). In bone, IL-6 is derived from osteoblasts and acts as an important regulator of osteoclastic development (104) and physiologically regulates bone metabolism (105). A recent study indicates that IL-6 induced osteogenesis of stem cells via signal transducer and activator of transcription factor (106). Mounting evidences have demonstrated that IL-6 directly promotes the formation of osteoclast through a RANKL-independent mechanism (101). It is recorded that IL-6 levels in periimplantitis was significantly higher than that in healthy subjects (107). Mice with IL-6 deficiency were also resistant to periodontal bone damage (108). Oral squamous cell carcinoma can not only produce IL-6 but also induce stromal cells to produce IL-6, and provide a suitable microenvironment for osteoclastogenesis (109). IL-6 is produced by oral cancer cells as a precursor protein that induced osteoclastic bone resorption and deficient bone formation through RANKL expression in stromal cells (110). It has been found that IL-6 appears to be a regulator of bone invasion and a direct critical driver of tumor growth and metastasis by oral cancer (111).

Interleukin 17 (IL-17)

IL-17 participates in both acute and chronic inflammatory responses, elicits similar host defense against extracellular bacterial infections and is crucial in inflammatory conditions including autoimmune diseases, cancer and metabolic disorders (112). IL-17 can stimulate osteoclastic bone resorption, suppress bone formation, and result in bone loss in osteoporosis (113, 114). IL-17 accelerates bone loss by promoting pro-osteoclastogenic cytokines accumulation containing TNF-α and RANKL produced by osteoblastic cells (115). Low expression of IL-17 inhibited the ability of bacteria in diabetic animals to induce inflammation and promote alveolar bone absorption in normal germ-free recipients (116). Interestingly, IL-17 expression is correlated with dendritic cells and increased in patients with chronic periodontitis (45). IL-17 can stimulate the synthesis of pro-inflammatory mediators including IL-6 and RANKL or indirectly promote periodontal bone loss (117). Rheumatoid arthritis is a systemic autoimmune disease which regulates inflammatory cytokines expression in the periodontal tissue, such as IL-1, TNF-α, IL-6, and IL-17 (118–121).

Interferon-Gamma (IFN-γ)

IFN-γ is the essential pro-inflammatory cytokines which is involved in the innate and adaptive immune responses (122). Data identify IFN-γ as the major effector cytokine driving pathogenesis in patients with immune-mediated bone diseases, such as postmenopausal osteoporosis and rheumatoid arthritis (53). IFN-γ plays dual effect in osteoclastogenesis. Substantial evidence demonstrated that IFN-γ enhanced bone resorption and led to bone loss under the pathological conditions (123, 124). In contrast, IFN-γ was a key negative regulator of osteoclastogenesis, and mediated the inhibition by IL-2 (BIL-2) in vitro (61, 125). Additional studies revealed that IFN-γ decreased serum calcium concentration and osteoclastic resorption in nude mice (126). It is reported that IFN-γ is effective in treating osteopetrosis by directly suppressing osteoclast differentiation but indirectly promoting bone resorption (127). Therefore, IFN-γ indirectly enhances osteoclastic factors via stimulating immune responses, otherwise, the lack of IFN-γ decreases alveolar bone loss in mice (128).

CONCLUSION AND PROSPECT

Destruction of bone homeostasis caused by immune dysfunction provides a clue to seek the therapeutic targets through osteoimmunology. Over the past decade, osteoimmunology plays a vital role in maintaining an adequate pool of cytokine and circulating immune cells to protect bone homeostasis. It provides a new inter-discipline to understand the relationship between the immune and the skeletal systems. Thus, a better understanding of the nexus between the immune and the skeletal systems should be at heart of future research in the area. Research in the field of osteoimmunology would pave path for novel therapeutics for treating bone losses resulted from different inflammation.

AUTHOR CONTRIBUTIONS

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