Chapter 23
The Role of Th1 Cytokines on Mechanical Loading-Induced Osteoclastogenesis and Bone Resorption

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Abstract Mechanical loading exerts important effects on the skeleton by controlling bone mass and strength. Osteoclasts are required for bone resorption and remodeling. Two cytokines are required for osteoclast formation: macrophage colony-stimulating factor and receptor activator of nuclear factor kappa-B ligand (RANKL). Tumor necrosis factor-α (TNF-α) has also been recognized as an important factor for osteoclastogenesis. It has previously been reported that interleukin (IL)-12 and IL-18, and interferon gamma (IFN-γ), which are type 1T helper cell (Th1) cytokines, inhibited RANKL- and TNF-α-mediated osteoclastogenesis. It also been reported that TNF-α plays an important role in mechanical loading-induced osteoclastogenesis and bone resorption. Orthodontic tooth movement is a good model for exploring the mechanism underlying mechanical loading-induced bone changes. Orthodontic tooth movement in a mouse model was established, and we investigated whether Th1 cytokines such as IL-12 and IFN-γ inhibit osteoclastogenesis and bone resorption upon mechanical loading. The number of tartrate-resistant acid phosphatase (TRAP)-positive cells increased at the pressure side of the first molar. Conversely, the amount of tooth movement and the number of TRAP-positive cells at the pressure side in IL-12- and IFN-γ-injected mice was less than that of non-injected mice. The results suggested that IL-12 and IFN-γ might have an inhibitory effect on mechanical loading-induced osteoclastogenesis. In this review, we describe and discuss the effect of Th1 cytokines on mechanical loading-induced osteoclastogenesis and bone resorption.

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23.1 Osteoclast Differentiation

Bone resorption is controlled by osteoclasts. Osteoclasts differentiate from hematopoietic stem cells [1]. Several important factors for osteoclast differentiation have been recognized. The receptor activator of nuclear factor kappa-B ligand (RANKL) [2], also known as osteoclast differentiation factor (ODF) [3], osteoprotegerin ligand (OPGL) [4], and tumor necrosis factor (TNF)-related activation-induced cytokine (TRANCE) [5] have been found to be essential in osteoclast differentiation. Macrophage colony-stimulating factor (M-CSF) is also identified as essential for the proliferation and differentiation of osteoclast precursors [6]. Op/op mice, which lack functional M-CSF, show osteopetrosis and have no osteoclasts. This deficiency can be cured by injection of M-CSF [7]. RANKL has been identified as a ligand of the receptor activator of nuclear factor kappa-B (RANK), which is an immunoresponsive receptor on dendritic cells [2]. RANKL-deficient mice have severe osteopetrosis and show a complete deficiency of osteoclasts [8]. Conversely, it has been reported that TNF-α also mediates osteoclast formation in vitro [9–11] and in vivo [12, 13]. TNF-α can also form osteoclasts independent of RANKL in the presence of transforming growth factor beta (TGF-β) [14]. However, it has been reported in another group that TNF-α failed to induce the differentiation of osteoclasts without RANKL [15]. They suggested that a constitutive level of RANKL was necessary for TNF-α-mediated osteoclast formation. Further studies are necessary to clarify this aspect.

23.2 TNF-α-Mediated Osteoclast Formation

TNF-α is pleiotropic and has a variety of biological effects in a cell-specific manner. TNF-α is known to play a major role in host defense, and exerts proinflammatory activities through various cells, including mononuclear phagocytes, in which it is responsible for the activation of bactericidal and cytocidal systems [16, 17]. It has been reported that TNF-α induces osteoclast formation from M-CSF-dependent bone marrow-derived macrophages in vitro [10]. TNF-α induced osteoclast recruitment might be central to the pathogenesis of inflammatory disorders [18]. TNF-α is a known contributor to rheumatoid arthritis [19], periodontal diseases [20, 21], and postmenopausal osteoporosis [22]. The findings that TNF-α recognizes two receptors on cell surfaces, type 1 or p55 (TNFR1) and type 2 or p75 receptors (TNFR2), and that each receptor is capable of distinct intracellular signaling [23], has substantially deepened our understanding of the complex activities of this cytokine. Analysis of TNFR1- and TNFR2-deficient mice revealed that TNFR1 induces osteoclast differentiation, while TNFR2 inhibits osteoclast

differentiation [24]. The role of TNF-α signaling in osteoclastogenesis remains poorly understood, and further studies are needed to clarify the relationship between TNF-α and osteoclast differentiation.

23.3 Mechanical Force Loading-Induced Osteoclast Formation and Bone Resorption

Mechanical loading force affects the skeleton by controlling bone mass and strength [25]. Several in vivo experimental models have been reported that evaluate the effect of mechanical loading on bone metabolism. The following experimental animal models have been established: jumping [26, 27], treadmill running [26, 28], squatting [29], and swimming [30]. Assessing an orthodontic tooth movement model in vivo is beneficial to understand the mechanism of mechanical loading-induced bone remodeling [31–34]. The animal models used for orthodontic tooth movement were usually rats and mice [35–43]. Opportunities for the use of various gene-mutated mice including those with genes that regulate bone metabolism have increased, because molecular biology techniques have progressed. Therefore, the mice tooth movement models can provide an understanding of the molecular mechanisms involved not only in tooth movement but also in mechanical loading-induced bone remodeling. A nickel-titanium (Ni-Ti) coil spring to obtain a continuous force for tooth movement is suitable for exerting continuous orthodontic force in mice models [44, 45]. The process of orthodontic tooth movement occurs by repeated alveolar bone resorption on the pressure side and bone formation on the tension side of teeth [46]. In orthodontic tooth movement, there is an association between osteoclasts and bone resorption on the pressure side [47]. In mice models, bone resorption was recognized on the pressure side and tartrate-resistant acid phosphatase (TRAP)-positive multinuclear cells also appeared on this side.

Mechanical force is relevant to tooth movement via the biological responses of cells in the periodontal ligament, alveolar bone, and other periodontal tissues [48]. Several factors, specifically cytokines and hormones, are related to this process. An increase in the level of TNF-α in the gingival sulcus during orthodontic tooth movement in humans has been reported [49, 50]. TNF-α has been shown to be expressed in rat periodontal tissue during excessive orthodontic force application [51]. When tooth movement was applied to TNFR1- and TNFR2-deficient mice, the amount of tooth movement observed in TNFR2-deficient mice was less than that in the wild-type mice [44]. These results suggested that TNFR2 is important for orthodontic tooth movement. However, it has been reported that the analysis of the reaction to TNF-α using TNFR1- or TNFR2-deficient mice showed the induction of osteoclastogenesis in TNFR1-deficient mice, whilst the inhibition of osteoclastogenesis was observed in TNFR2-deficient mice [20]. These results are controversial. In the evaluation of the role of TNFR1 in osteoclast formation during orthodontic tooth movement, the number of osteoclasts in TNFR1-deficient mice

was found to be lower than that in wild-type mice [52]. To further investigate the role of TNFRs, tooth movement experiments using double-mutated mice for both TNFR1 and TNFR2 were performed. The experiment showed significant decreases in the amount of tooth movement in the double-mutated mice [45]. These results suggested that TNF-α is associated with orthodontic tooth movement. However, the relationship between orthodontic movement and TNF-α is not yet completely understood.

23.4 Effect of Interleukin (IL)-12 on Osteoclast Formation

IL-12 is one of the type 1T helper cell (Th1) cytokines. IL-12 has been recognized as playing an important role in host defense. It induces differentiation of native T cells into IFN-γ-producing Th1 cells that are resistant to infection [53]. IL-12 is a heterodimeric disulfide-linked 70-kDa protein consisting of 35- and 40-kDa subunits. It has previously been shown that IL-12 plays an important role in attaining the optimal level of cell-mediated immune response against intracellular pathogens [54]. IL-12 is produced by osteoblasts infected with *Staphylococcus aureus*, which is the most prevalent causative microorganism in osteomyelitis, a bone resorption disorder [55].

It has been shown that IL-12 inhibits osteoclast formation in the spleen cells of mice treated with M-CSF and RANKL [56]. It was found that the inhibitory effect of IL-12 depends on the presence of T cells among spleen cells. However, it has been reported that osteoclastogenesis induced by RANK/RANKL interaction decreased in the presence of IL-12 by a T-cell-independent mechanism in vitro [57], and TNF-α-induced osteoclastogenesis was also inhibited through induction of apoptosis mediated by the interaction of the IL-12-induced Fas ligand (FasL) and TNF-α-induced Fas in vitro [58]. IL-12 and IL-18 inhibited TNF-α-mediated osteoclastogenesis by up-regulating FasL synergistically [59]. IL-18 is also an important Th1 cytokine. It has been reported that IL-18 can also inhibit osteoclast formation in spleen cell cultures in vitro and that the IL-18-mediated inhibition of osteoclast formation is also T cell dependent [56].

The target cells of IL-12 have been shown to be T cells [54], natural killer (NK) cells [60], natural killer T cells, B cells [61], dendritic cells [62], and macrophages [63]. It has been reported that IL-12 influences non-adherent cells in bone marrow cell cultures and induces FasL expression in non-adherent cells. The results suggested that adherent cells, such as dendritic cells and macrophages, are not target cells [54]. In addition, when bone marrow macrophages were co-cultured with T cells isolated from among spleen cells in the presence of M-CSF, TNF-α, and IL-12 in vitro, apoptotic alterations were not observed [58]. In the study, when whole bone marrow cells from T-cell-deficient nude mice were cultured in the presence of M-CSF, TNF-α, and IL-12, the cells underwent apoptosis similar to those of wild-type mice [58]. These results also

suggest that T cells may not be target cells for IL-12 in this case. However, additional experiments are necessary to clarify the target cells for IL-12.

The effect of IL-12 on mechanical tooth movement in mice has been reported. Mechanical tooth movement, in which a Ni-Ti closed coil spring was inserted between the upper incisors and the first molar in mice, was used. IL-12 was injected into a local site adjacent to an upper molar during tooth movement. After 12 days, the distance of tooth movement was measured. The number of osteoclasts, which are TRAP-positive cells, were counted in a histological section. Tooth movement was inhibited when IL-12 was localized. The number of TRAP-positive cells was reduced in IL-12-treated mice [64].

Root resorption is a disagreeable phenomenon of orthodontic treatment, which may present at the dentinal and cemental areas of the tooth root surface, and is a serious problem for the orthodontist [65]. Even under normal conditions, it is possible to cause root resorption during orthodontic tooth movement [66–68]. Inhibition of root resorption is hopeful for orthodontists. In recent years, there have been studies investigating the use of medicine for future clinical application to prevent root resorption. It has been reported that bisphosphonates inhibit root resorption [69, 70]. In addition, it has been shown that osteoprotegerin inhibits root resorption more effectively than bisphosphonates [71]. Furthermore, the inhibitory effect on root resorption by amelogenin [72], bisphosphonates, and anti-c-Fms antibodies has also been reported [73, 74]. Root resorption was recognized in this tooth movement model. The root resorption area was measured using a scanning electron microscope. The root resorption area was reduced in IL-12-treated mice. In TdT-mediated dUTP-biotin nick end-labeling (TUNEL) assays, many apoptotic cells were seen on the pressure side in IL-12-treated mice. These findings indicate that IL-12 inhibits mechanical tooth movement and root resorption in orthodontic tooth movement. These results might be the outcome of apoptosis induced by IL-12.

23.5 Effect of IFN-γ on Osteoclast Formation and Bone Resorption

IFN-γ contributes to T-cell-mediated regulation of immune responses and is secreted by Th1 cells, cytotoxic T cells, dendritic cells, and NK cells [75]. In addition, IFN-γ has been recognized as an activator of macrophages because of the induction both of nitric oxide production and major histocompatibility complex presentation in macrophages, and exhibits antiviral and antibacterial activity [76].

The effect of IFN-γ has been recognized as suppression of osteoclast formation by inhibition of RANKL signaling via degradation of TNF receptor-associated factor 6 [77]. Furthermore, the bone resorption in collagen-induced arthritis was enhanced in IFN-γ-deficient mice [78]. These data indicated that IFN-γ inhibited osteoclast formation and bone resorption. However, it has been reported that IFN-γ

indirectly stimulates osteoclast formation via antigen-driven T-cell activation [79]. Therefore, the role of IFN-γ in osteoclast formation is still unclear.

It has been reported that IFN-γ directly inhibits osteoclastogenesis induced by TNF-α stimulation and accelerates apoptosis mediated by Fas/FasL signals. IFN-γ directly interrupted TNF-α-induced osteoclast formation as revealed with a decreased number of osteoclasts and messenger ribonucleic acid (mRNA) levels of nuclear factor of activated T cells, cytoplasmic 1 (NFATc1), which is a gene essential for osteoclast formation, in cultured bone marrow macrophages. Apoptotic findings of cultured cells were evaluated by accelerated nuclear fragmentation in osteoclast precursor cells. Fas mRNA levels in bone marrow cells were stimulated by TNF-α. FasL mRNA levels in a bone marrow culture with IFN-γ was increased. Furthermore, IFN-γ inhibited osteoclastogenesis in response to TNF-α treatment in vivo. IFN-γ inhibited TNF-α-induced osteoclastogenesis in mice with T cells blocked by anti-CD4 and anti-CD8 antibodies [80]. These results suggested that IFN-γ directly inhibits osteoclastogenesis, and induces cell apoptosis by Fas/FasL signaling, leading to the indirect regulation of bone resorption. This might occur as a protective role against bone destruction at an inflammation site.

The cellular responses in periodontal tissue, including the alveolar bone, periodontal ligament, and other periodontal tissues, during mechanical force-driven tooth movement are mediated by interactions between various factors such as cytokines and hormones [48, 81]. In a rat tooth movement model, IFN-γ is expressed on the pressure side of teeth [82]. IFN-γ, which increases trabecular bone volume, has been evaluated histomorphometrically during orthodontic tooth movement in rats [83]. Therefore, these results suggest that IFN-γ plays an important role in orthodontic tooth movement. However, there are few studies on the effect of IFN-γ on tooth movement. The effect of IFN-γ on mechanically loaded tooth movement in a mouse model has been reported. A Ni-Ti closed coil spring was inserted between the upper anterior alveolar bone and the upper left first molars in mice. The relationship between local IFN-γ mRNA levels and orthodontic tooth movement was evaluated. In other experiments, IFN-γ was injected to each first molar every other day during tooth movement. After 12 days, the amount of tooth movement was measured. The number of osteoclasts at the pressure side of each experimental tooth was assessed. Local IFN-γ mRNA expression increased with orthodontic tooth movement in mice. The number of osteoclasts increased on the pressure side of the first molar. In contrast, the distance of tooth movement and the number of osteoclasts on the pressure side in IFN-γ-injected mice were less than those of control mice. IFN-γ expression was increased in experimental tooth movement. Furthermore, IFN-γ could inhibit mechanical force-induced osteoclast formation and tooth movement. These results suggest that IFN-γ might be useful in controlling orthodontic tooth movement, because IFN-γ inhibited the action of progressive osteoclast formation during orthodontic tooth movement [84]. These results lead us to conclude that IFN-γ induction is able to inhibit mechanical force-loaded osteoclast formation, consequently inhibiting orthodontic tooth movement.

23.6 Conclusions

It has been reported that many types of cytokines are expressed during mechanical loading of the periodontal ligament. TNF-α is an important molecule in mechanical loading force-induced osteoclast formation in the periodontal ligament during orthodontic tooth movement. Therefore, it is important to study the relationship between TNF-α-induced osteoclast formation and the cytokines expressed during mechanical loading. Th1 cytokines inhibited osteoclast and odontoclast formation during mechanical loading in the periodontal ligament. There is a possibility that local injection of Th1 cytokines might be a useful tool to enhance the anchorage site and control the rate of tooth movement during orthodontic treatment, as well as prevent relapse after orthodontic treatment. Moreover, local injection of Th1 cytokines might be a useful tool in reducing root resorption, particularly for high-risk teeth. However, further studies are required to fully understand the relationship between mechanical loading-induced osteoclast formation and the effect of cytokines.

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