The Role of Osteocytes in Inflammatory Bone Loss

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Osteoimmunology investigations to-date have demonstrated the significant interactions between bone surface cells, osteoclasts and osteoblasts, and immune cells. However, there is a paucity of knowledge on osteocytes, cells embedded in the bone matrix, and their role in inflammation and inflammatory bone loss. Osteocytes communicate through various mechanisms; directly via dendritic processes and through secretion of proteins that can influence the formation and activity of osteoblasts and osteoclasts. Some osteocyte proteins (e.g., interleukin-6 and RANKL) also have roles within the immune system. In the context of mechanical loading/unloading, the regulatory role of osteocytes is well understood. More recent data on osteocytes in various inflammatory models suggest they may also aid in orchestrating inflammation-induced changes in bone turnover. In inflammatory conditions, osteocytes express multiple pro-inflammatory cytokines which are associated with increases in bone resorption and declines in bone formation. Cytokines are known to also influence cell population growth, maturation, and responsiveness via various signaling modalities, but how they influence osteocytes has not been greatly explored. Furthermore, osteocytes may play regulatory roles in orchestrating bone's response to immunological changes in inflammatory conditions. This review will address what is known about osteocyte biology in physiological conditions and in response to varying immunological conditions, as well as highlight key areas of interest for future investigations.

Keywords: osteocyte, cytokines, inflammation, tumor necorosis factor (TNF), sclerostin

Bone continually adapts to its internal and external environment, undergoing formation and resorption to maintain bone mass. Bone loss is an imbalance of bone formation and resorption which occurs in many conditions including disuse, aging, and chronic inflammatory conditions such as inflammatory bowel disease (1), rheumatoid arthritis (2), psoriasis (3), and systemic lupus erythematosus (4). While comparatively less studied to disuse or age-related bone loss, inflammatory conditions are also associated with increased fracture incidence (4–8). Multiple other conditions with known inflammatory components are also associated with bone loss and increased bone fragility including type 2 diabetes (9), chronic kidney disease (10), spinal cord injury (11), and aging-related osteoporosis (12). While the role of immune factors and inflammation on various bone cells (osteoblasts, osteoclasts, stromal cells, marrow immune cells, bone precursor cells) have been extensively researched (13), the osteocyte response to inflammatory stimuli has been comparably less investigated. However, osteocytes have increasingly become appreciated as key regulators of both osteoclasts and osteoblasts, orchestrating changes in bone turnover.

INTERACTIONS OF THE IMMUNE SYSTEM AND BONE

The immune system interacting with bone physiology is being elucidated in the burgeoning field of osteoimmunology. For example, cytokines are signaling proteins released by various cell-types that modulate the direction of an immune response but can also influence the cellular phenotype of immune and parenchymal cells. In general, pro-inflammatory cytokines are grouped as Th_1, while anti-inflammatory cytokines as Th_2 (14). Notably, cytokines can interact with bone cells leading to increased bone resorption and decreased bone formation that, over time, leads to inflammatory bone loss (15). Key Th_1 cytokines include TNF-α and IL-1β, both stimulators of osteoclastogenesis (16–19). Additionally, these cytokines increase the production of RANKL, a key osteoclastogenesis regulator (20–22). TNF-α can also increase production of OPG, the decoy receptor for RANKL (23). IL-6 has equivocal roles in bone physiology, but, with TNF-α, IL-6 synergizes to stimulate osteoclasts and increase production of RANKL and OPG (21). IL-17 is also a potent stimulus for osteoclastogenesis (24) and sensitizes osteoclast pre-cursors to RANKL (25).

In addition to stimulating an increase in osteoclasts and bone resorption, Th_1 cytokines interact with osteoblast development and function. For example, TNF-α inhibits osteoblast genes and differentiation factors (e.g., *RUNX2*), and reduces bone collagen synthesis (20, 26–28). TNF-α also inhibits the anabolic effects of IGF-I on osteoblasts (29) and induces osteoblast apoptosis (20, 30). Interleukin-1β (IL-1β) has been shown to also suppress bone formation (31). In addition to osteoblasts and osteoclasts, other cell types residing in the bone environment such as bone marrow stromal cells and osteoblast/osteoclast-precursor cells can be influenced in their function by cytokines (32, 33). This illustrates the critical role of immunological factors, such as Th_1 cytokines, in bone biology.

Th_2 cytokines, such as IL-4 and IL-10, are less well understood in the context of bone physiology. In cell culture models, both IL-4 and IL-10 have been shown to inhibit osteoclasts and reduce RANKL production (34–37). Another cell culture study found both IL-4 and IL-13 increased OPG mRNA expression; thus, decreasing RANKL mediated osteoclastogenesis *in vitro* (38). Furthermore, IL-10 transgenic knockout mice have low bone mass and increased fragility which alludes to an influential role of IL-10 in regulating bone turnover (39). There exist many other cytokines within the Th_1 and Th_2 classes and other subsets (Th_9, Th_{17}, Th_{22}, T_{fh}) that have roles not yet delineated in bone physiology, highlighting areas of future research. Finally, the interaction of these cytokines with osteocytes has been minimally investigated.

OSTEOCYTE BIOLOGY

Osteocytes are the longest living bone cell, making up 90–95% of cells in bone tissue in contrast to osteoclasts and osteoblasts making up ∼5% (40). Osteocytes form when osteoblasts become buried in the mineral matrix of bone and develop distinct features. Residing within the lacuna of the mineralized bone matrix, osteocytes form dendritic processes that extend out from their cell bodies into spaces known as canaliculi. Through these dendritic processes, osteocytes form networks interfacing with other osteocytes, cells on bone surfaces, and the marrow (40). Through these communication networks, osteocytes sense the local and systemic environment within the bone.

Osteocytes also coordinate the actions of osteoblasts and osteoclasts via several mechanisms. First, osteocytes express and release proteins that signal to osteoblasts, osteoclasts, and other bone-residing cells to respond to environmental changes. Osteocytes express important factors for the maintenance of mineral homeostasis including SOST, Phex, DMP1, and FGF23 (41). Sclerostin, the protein encoded by the SOST gene, is an antagonist of the Wnt/β-catenin system, with increased sclerostin expression leading to a suppression of bone formation (42–44). Osteocytes also produce RANKL and OPG, critical regulators of osteoclastogenesis. While osteoblasts and other bone-residing immune cells also produce RANKL, it is now appreciated that RANKL synthesized by osteocytes is a significant source of RANKL driving osteoclast formation for bone remodeling (45–47). Additionally, osteocyte apoptosis signals to increase osteoclast activity driving targeted bone resorption (41, 48, 49). Elucidating osteocyte function in the context of osteoimmunology may provide further insight to the imbalance of resorption vs. formation seen in inflammation-induced bone loss.

THE ROLE OF OSTEOCYTES IN ADAPTATIONS TO MECHANICAL STRAINS

In the past few decades, the central role of osteocytes in response to mechanical strains has been explored and identified. Osteocytes sense mechanical strains through fluid flow shear stress through the lacuna-canalicular network and changes in interstitial hydrostatic pressure (50–52). Decreased mechanical strains also induce osteocyte apoptosis leading to decreased bone mass and strength (53, 54). Some preliminary evidence suggests that high mobility group box 1 (HMGB1), an alarmin (55), may be released during osteocyte apoptosis thereby triggering RANKL and other immune factors (56). It is unknown what other immune-related factors may be released during apoptosis and the signaling cascades that follow.

Mechanosensory signals also trigger osteocytes to release various proteins that impact bone turnover. RANKL and OPG are also known to be mechanosensitive (57) and mice lacking osteocyte RANKL are protected from disuse-induced bone loss (46). Furthermore, unloading-induced osteocyte apoptosis initiates an increase in osteocyte RANKL (54). Prevention of osteocyte apoptosis in animal models of unloading mitigates increases in osteocyte RANKL (54, 58). Disuse is also characterized by elevated osteocyte sclerostin in conjunction with decreased bone formation rate (59, 60). Other mechanosensitive osteocyte proteins include insulin-like growth factor-I (IGF-I) and IL-6 which both are upregulated with loading (60–63). The role of osteocytes in the mechanosensory capabilities

of bone highlight the important role these cells play in bone adaptations to the environment. Some osteocyte proteins known to have mechanosensory roles such as RANKL and IL-6 are also signaling molecules in the immune system and play key roles in inflammatory processes. This suggests that other cytokines may perhaps have similar dual roles responding under conditions of loading/unloading and under conditions of inflammation.

OSTEOCYTES AND CYTOKINES

Burgeoning research has shown cytokines directly impact osteocyte apoptosis and cause the release of cytokines that influence bone turnover. In cell culture models, osteocyte apoptosis can be induced by both TNF-α and IL-1β (64–66). A mouse infectious osteomyelitis model lead to increases in osteocyte apoptosis, as well as elevations in gene expression of TNF-α, IL-1β, IL-6, and IL-17 in the femur; the same treatment in TNF-α deficient mice resulted in fewer apoptotic osteocytes (67). We previously demonstrated in rats with inflammatory bowel disease decreased osteocyte density and increased apoptosis concurrent with elevated osteocyte TNF-α (68, 69). Therefore, one mechanism of increased bone resorption in inflammatory conditions is through the direct effect of pro-inflammatory cytokines on osteocyte apoptosis which, in turn, increases osteoclastic driven resorption.

Osteocytes also express pro-inflammatory cytokines. TNF-α is expressed in the MLO-Y4 osteocyte-like cell culture line (70, 71), as is IL-6 (61). Cultured human trabecular bone chips expressing osteocyte-specific genes also express TNF-α, IL-6, IL-1β, and IL-8 (72). Other cell culture osteocyte lines have increased expression of pro-inflammatory cytokines with exposure to monosodium urate crystals (73), Brucella abortus infection (74) and orthopedic implant materials (75). Immunohistochemical analysis of rat bones demonstrate elevated osteocyte TNF-α, IL-6, and IL-17 in various inflammatory conditions (68, 69, 76). Therefore, osteocytes express cytokines that can increase osteoclastogenesis and inhibit osteoblast formation or activity.

Osteocytes themselves respond to circulating pro-inflammatory cytokines influencing their cytokine expression. For example, exposing MLO-Y4 osteocytes in culture to IL-17 increases expression of TNF-α (71). In cultured human bone chips with osteocyte-enriched cells, gene expression of TNF-α, IL-1β, and IL-6 is elevated upon treatment combinations of TNF-α, IL-1β, and IL-6 (72). Based on the supporting data from these *in vitro* studies, cytokines influence osteocytes in a positive-feedback mechanism leading to even greater cytokine expression. This would suggest osteocytes may amplify an inflammatory bone state resulting in increased production of factors altering bone turnover and increasing bone loss.

Many cytokines also alter osteocyte signaling proteins. Osteocyte-to-osteoclast signaling is enhanced by multiple pro-inflammatory cytokines largely through RANKL signaling. Culture media from IL-1β-treated MLO-Y4 cells increased osteoclastogenesis *in vitro* (77). Furthermore, blocking IL-17A

prevented the increase in osteocyte RANKL due to continuous parathyroid hormone exposure (78).

MLO-Y4 cells treated with IL-6/IL-6R and co-cultured with osteoclast precursors also results in increased osteoclastogenesis due to elevated RANKL (79). Furthermore, RANKL-positive osteocytes are elevated in animal models of inflammatory conditions including periodontitis (80–82), spinal cord injury (76), and inflammatory bowel disease (68, 69). Furthermore, in rat models of inflammatory bowel disease and spinal cord injury, RANKL-positive osteocytes were associated with increases in osteoclast surfaces (68, 76). In contrast to RANKL, OPG is less well understood in conditions of inflammation. Treatment of cultured human osteocytes- with a combination of IL-1β, TNF-α, and IL-6 upregulates OPG (72). In rodent inflammatory bowel disease and spinal cord injury models, OPG-positive osteocytes were elevated (68, 69, 76). Therefore, while the exact role of OPG is not known, it is known that inflammatory cytokines regulate osteoclastogenesis in part through osteocyte-mediated RANKL/OPG signaling.

Inflammatory signals also influence osteocyte proteins controlling bone formation. Wnt proteins are key mediators of osteoblastogenesis and govern the formation of the skeletal development. Both sclerostin and Dickkopf-related-1 (Dkk-1) inhibit the Wnt signaling pathway in bone. Dkk-1 is upregulated by TNF-α and blockade against Dkk-1 in transgenic mice with inflammatory arthritis prevents bone loss (83); however, osteocyte-specific deletion of Dkk1 did not protect against inflammatory arthritis-induced bone loss (84). Interestingly, Dkk1 expression was inhibited in osteoblast cell culture treated with IL-17A; whether this is also true in osteocytes is unknown (85). Sclerostin has also been shown to bind to LRP5/6 and inhibit Wnt signaling *in vitro* and *in vivo* (42, 86), and be transcriptionally activated by TNF-α (87). In human osteocyte-enriched cell cultures, serum from rheumatoid arthritis patients, IL-1β alone, and a combination of IL-1β, TNF-α, and IL-6 all increased SOST expression (72). Furthermore, osteocyte sclerostin is elevated in animal models of high fat diets with elevated serum and osteocyte TNF-α (87), inflammatory arthritis (83), periodontitis alveolar bone (81, 82), spinal cord injury (76), and inflammatory bowel disease (68, 69). Beyond direct effects of pro-inflammatory cytokines on osteoblasts and bone formation, the inflammation-induced elevation of inhibitors of bone formation contributes to a state of low bone formation.

While outside the scope of this review, additional osteocyte proteins involved in mineral homeostasis and metabolism are influenced by inflammatory signals. Fibroblast growth factor 23 (FGF23), a phosphate regulator synthesized by osteocytes, has increased expression in inflammatory conditions (72, 88, 89). IL-17A has been shown to decrease various genes of osteocyte proteins involved in mineral metabolism including Dmp1 and Phex (90). Therefore, it is clear that osteocytes respond to inflammatory signals through various mechanisms including increased expression of cytokines and altered expression of regulatory proteins.

MECHANOSENSING AND INFLAMMATORY SIGNALS

Crucial to osteocyte function is sensing and responding to bone interstitial fluid shear stress and mechanical strains. Furthermore, there is some overlap in signaling proteins between inflammation and mechanosensing (sclerostin, RANKL, OPG, etc.). What is not fully understood is if mechanosensing is tied with osteocyte inflammatory responses and vice versa. Utilizing pulsatile fluid flow in MLO-Y4 cells, TNF-α, and IL-1β treatment inhibits fluid flow-induced increases in calcium uptake and nitric oxide release indicating a potential blunting of the osteocyte response to mechanical strains (65). Another investigation found media from MLO-Y4 cells cultured with IL-1β induced osteoclastogenesis, while IL-1β-cultured cells that also underwent pulsatile fluid flow prevented osteoclastogenesis (77). Pulsatile fluid flow reduced MLO-Y4 expression of TNF-α- and IL-17A-induced increases in TNF-α and RANKL (71), inhibited TNF-α-induced osteocyte apoptosis (66), and increased IL-6 production in osteocyte cultured cells (61).

With aging, osteocytes develop morphological adaptations and changes in the lacunocanalicular system that may impair their mechanosensory function and ability to communicate (91, 92). In addition, osteocytes of aged mice also express a senescence-associated secretory-phenotype, expressing multiple pro-inflammatory cytokines including IL-17A, IL-1A, and IL-6, likely contributing to age-related bone loss (93). Prevention of the pro-inflammatory secretome of senescent cells in aged mice with a JAK inhibitor improved bone mass and strength (94). It has been hypothesized that exercise to increase mechanical strain on bone could improve the senescent phenotype in aging (95). It remains to been seen whether a lack of mechanical loading and a lack of adequate mechanosensory ability or pro-inflammatory senescent markers occurs first during aging in osteocytes.

To our knowledge, there are no investigations directly assessing the influence of mechanical loading on osteocyte-related proteins in animal models of inflammatory conditions to determine the mechanical loading effects on osteocyte inflammatory changes. However, it is possible that in inflammatory pathologies, such as spinal cord injury where both chronic systemic inflammation and disuse are present, the inflammatory status with the lack of mechanical loading on osteocytes could exacerbate bone loss. Further work needs to be done on the interaction of inflammation and mechanical strains in osteocytes.

FUTURE DIRECTIONS

With the accumulating knowledge of the role of osteocytes in inflammatory bone loss, future areas of interest may include

therapeutically targeting osteocytes in inflammatory bone loss conditions. In inflammatory conditions, bone-specific treatments like bisphosphonates, anti-RANKL, and anti-sclerostin, all improve bone outcomes, but have no effect on inflammatory measures (96–98). Anti-inflammatory treatments, like anti-TNF, may improve bone mass (99), but potentially have negative side effects (100, 101). Therefore, viable treatments for inflammatory conditions are still needed. By directly impacting osteocytes via senolytic treatment or a JAK inhibitor in aged mice prevented the inflammatory senescent osteocyte phenotype (94). Additionally, in rodent models of inflammatory bowel disease, a soy protein diet and treatment with exogenous irisin decreased the inflammatory status of osteocytes and improved bone turnover (69, 102). Other anti-inflammatory treatments that improve bone in inflammatory conditions, like resolvin E1 (103), need to be examined for their impact on osteocytes. Furthermore, low-grade inflammation may be beneficial in some conditions like fracture healing (104). The role of other cytokines in bone physiology still needs to be elucidated as well as the relative contribution of osteocytic pro-inflammatory cytokines vs. those from other cell types in the marrow. Finally, the magnitude and duration of mechanical forces that could influence osteocyte-immune crosstalk has yet to be examined.

CONCLUSIONS

Osteocytes play a central role in orchestrating changes in bone turnover. In this review, we present literature that supports an overlap between classical osteocyte regulatory proteins with known mechanosensory functions (RANKL, OPG, sclerostin, etc.) and immune factors that directly impact osteocytes and their communication with osteoblasts and osteoclasts. Pro-inflammatory signals stimulate osteocyte apoptosis, increase osteocyte cytokine production, and alter osteocytic proteins controlling bone turnover. Therefore, osteocytes are key players in inflammatory bone loss. This indicates that osteocytes may be targets for preventing/treating inflammatory bone alterations.

AUTHOR CONTRIBUTIONS

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