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T cells, osteoblasts, and osteocytes: interacting lineages key for the bone anabolic and catabolic activities of parathyroid hormone

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Abstract

Osteoimmunology is field of research dedicated to the study of the interactions between the immune system and bone. Among the cells of the immune system that regulate bone turnover and the responsiveness of bone cells to calciotropic hormones are bone marrow T lymphocytes. T cells secrete osteoclastogenic cytokines such as RANKL and TNF- α , as well as factors that stimulate bone formation, one of which is Wnt10b. In addition, T cells regulate the differentiation and life span of stromal cells and their responsiveness to parathyroid hormone (PTH) via costimulatory molecules expressed on their surface. The conditioning effect of T cells on stromal cells (SCs) is inherited by the osteoblastic and osteocytic progeny of SCs. As a result, osteoblastic cells of T cell-deficient mice have functional characteristics different from corresponding cells of T cell-replete mice. These differences include the ratio of RANKL/OPG produced in response to continuous PTH treatment, and the osteoblastogenic response to intermittent PTH treatment. This article reviews the evidence indicating that the effects of parathyroid hormone are mediated not only by osteoblasts and osteocytes but also by T cells.

Keywords

T cells; PTH; osteoblasts; osteocytes; stromal cells; bone

Introduction

Osteoimmunology is a discipline dedicated to the study of the interactions between the immune system and bone. Clinicians who have reported an association between inflammation and bone loss and increased fracture risk have long suspected the existence of important interaction between immune and bone physiology.^{1–3} For example, low-grade systemic inflammation, indicated by moderately elevated serum levels of high sensitivity C-reactive protein, associates with low bone density, elevated bone resorption, and increased fracture risk.^{1, 2, 4} At the cellular level it is now well established that during inflammation

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osteoclastic bone resorption is driven by inflammatory cytokines produced by activated T cells.⁵ The discovery that the RANKL–RANK system plays a pivotal role in both adaptive immunity and osteoclastogenesis provided molecular evidence that has firmly established a link between the immune system and bone. The subsequent finding that RANKL produced by T cells contributes to the bone loss observed in adjuvant-induced arthritis⁵ provided the first incontrovertible evidence for the capacity of some T cell lineages to cause bone loss. Initially recognized as a lineage capable of stimulating bone resorption, bone marrow (BM) T cells are known to also regulate bone formation. T cells regulate bone homeostasis via direct interactions with bone cells mediated by cell surface molecules, and by releasing cytokines and Wnt ligands. This article focuses on the role of BM T cells, osteoblasts, and osteocytes, and their interactions in the mechanism of action of PTH in bone.

T lymphocytes relevant to bone

Bone remodeling is regulated by interactions between bone cells and BM cells. Therefore, the lymphocytes critical for bone remodeling are those that home and reside in the BM. Accounting for only ~5 % of total BM cells, T cells are highly mobile and distributed among the stroma and parenchyma.^{6, 7} T cells are a heterogeneous population that includes $\alpha\beta$ and $\gamma\delta$ T cell populations. These two families differ in the chains ($\alpha\beta$ or $\gamma\delta$) forming their T cell receptor. $\alpha\beta$ T cells are divided into naive and memory $CD4^+$ and $CD8^+$ T cell populations. Naive $CD4^+$ cells differentiate into T_H1 , T_H2 , T_H17 , and regulatory T (T_{reg}) effector cells. Some T cells express the cell surface marker of natural killer (NK) cells, and are thus referred to as NKT cells.

The BM is regarded as a niche for the recruitment and retention of central memory $CD8^+$ cells.⁶ The BM as a lower proportion of $CD4^+$ cells as compared to peripheral blood, and in fact the $CD4^+/CD8^+$ ratio is <1 in the BM, while is typically 2:1 in other lymphoid organs.⁶ Memory $CD8^+$ cells have a higher activation state than peripheral blood T cells, and consequently secrete relatively high levels of effector cytokines. Disease state may further alter the number and phenotype of BM T cells, leading to a further exacerbation of the pathological process. For example, postmenopausal women with osteoporotic fractures have a higher proportion of TNF- α -producing $CD8^+$ cell as compared to age-matched control women^{9, 10} Some T cell lineages (e.g., T_H17 cells) have been recognized as cells capable of stimulating bone resorption, while others (e.g., T_{reg} cells) have been shown to inhibit osteoclastogenesis; but there is increasing recognition that T cells may function as activator of bone formation, as they can secrete Wnt ligands that activate Wnt signaling in osteoblastic cells.¹¹⁻¹³

The net effect of T cells depends on their activation state and by their specific phenotype. Activated $CD4^+$ and $CD8^+$ T cells promote bone loss in inflammatory diseases such as rheumatoid arthritis⁵ and periodontitis.¹⁴ Activated T cells also play a critical role in bone cancers¹⁵ and in post-menopausal osteoporosis.^{9, 10} Conversely, resting $CD4^+$ T cells may contribute to dampening of bone resorption *in vivo*.¹⁶ Accordingly, T cell-deficient mice have significantly increased bone resorption and reduced bone density, as compared to controls.¹⁷ Activated conventional $CD4^+$ and $CD8^+$ T cells and T_H17 cells regulate bone homeostasis via surface bound molecules that bind to cognate molecules expressed in

osteoblasts and their stromal cell (SC) precursors¹⁸ and by releasing osteoclastogenic cytokines such as RANKL, TNF- α , IL-1, IL-6, and IL-17. Activated CD8⁺ T cells can also release Wnt ligands such as Wnt10b¹¹⁻¹³ that activate Wnt signaling, thus promoting osteoblastogenesis. Naive helper T cells differentiate into several lineages including T_H1, T_H2, and T_H17 cells in response to cytokines secreted by bone cells spontaneously or in response to calicotropic hormones. By regulating T cells differentiation via their cytokines, bone cells lead to expansion of mature T cells populations that further regulate bone homeostasis²⁰.

The most osteoclastogenic subsets of T cells are T_H17 cells.²¹ These cells are defined by their capacity to produce the cytokine IL-17. Therefore, CD4⁺ IL-17⁺ cells are referred to as T_H17 cells. T_H17 cells are constitutively present at mucosal surfaces, especially in the intestinal lamina propria,²² and are relatively frequent in the BM^{23, 24} because bone is a large reservoir of TGF- β and IL-6, factors essential for T_H17 cell differentiation. T_H17 cells play a pivotal role in the bone loss of inflammatory conditions such as psoriasis, rheumatoid arthritis, periodontal disease, and inflammatory bowel disease (IBD).^{25, 26} T_H17 cells potently induce osteoclastogenesis by secreting IL-17, RANKL, TNF- α , IL-1, and IL-6, along with low levels of IFN- γ .²⁷⁻²⁹ IL-17 stimulates the release of RANKL by osteoblasts and osteocytes^{21, 30} and potentiates the osteoclastogenic activity of RANKL by upregulating RANK.³¹ IL-17 provides an important connection between T cells and osteocytes, as this T cell cytokine regulates osteocytic RANKL production,³⁰ a key effect of PTH on osteocytes.^{32, 33} T_H17 cells have been linked to postmenopausal osteoporosis because the differentiation of T_H17 cells is induced by ovariectomy (ovx),³⁴ while the bone loss induced by ovx is prevented by silencing of IL-17R³⁵ and treatment with anti-IL-17.³⁶ Importantly, elevated levels of IL-17 have been found in postmenopausal women with osteoporosis.³⁷⁻³⁹

The most bone-sparing population of T cells are T_{reg} cells, a suppressive population of CD4⁺ T cells defined by the expression of the transcription factor FoxP3 and the ability to block conventional T cell proliferation and production of effector cytokines.⁴⁰ T_{reg} cells form when naive CD4⁺ cells are exposed to antigen in the presence of TGF- β . Defects in T_{reg} cell numbers and/or activity have been implicated in several chronic inflammatory diseases. As a result, there has been a recent explosion in research investigating the potential to manipulate T_{reg} cells for clinical purposes⁴¹⁻⁴⁵. It is now clear that T_{reg} cells regulate osteoclast (OC) formation⁴⁶⁻⁴⁹ and prevent ovx-induced bone loss.⁵⁰ T_{reg} cells blunt bone resorption^{47, 51} through the secretion of IL-4, IL-10, and TGF- β .⁴⁹ Estrogen increases the relative number of T_{reg} cells.⁵² It is interesting to note that the cytokines produced by T_{reg} cells to repress effector T cells also possess a strong anti-osteoclastogenic activity. Intravital microscopy studies have disclosed that T_{reg} cells are not randomly distributed in the BM but rather reside in close proximity with endosteal bone surfaces and osteoclasts (OB).⁵³ Osteoclasts selectively recruit and activate CD8⁺ T cells,^{54, 55} which express CD25 and FoxP3 and therefore are defined as osteoclast-induced regulatory CD8 T cells.⁵⁶ Moreover, a recent report has disclosed that although RANKL directly stimulates osteoclasts to resorb bone, at lower doses this cytokine also controls osteoclast ability to induce regulatory T cells, an important negative feedback loop. Attesting to relevance, ovariectomized mice treated with low-dose RANKL exhibit CD8⁺ T_{reg} cells that suppress bone resorption, decrease the levels of inflammatory/osteoclastogenic cytokines, and stimulate bone

formation. These studies suggest the existence of a novel regulatory loop, whereby osteoclasts and the osteoclast-inducing factor RANKL induce T_{reg} cells, and then the T_{reg} cells blunt osteoclastic bone resorption.⁵⁷

T cells make use of several mechanisms to regulate bone cells. First, they are armed with surface costimulatory molecules that bind their counter receptors expressed in bone cells. For example, T cells express RANKL and CD40L, which activate the cognate receptors RANK and CD40 in osteoclast precursors and osteoblastic cells, respectively.^{18, 20, 58, 59} CD40L, also known as CD154, exerts its effects by binding to CD40⁶⁰ and several integrins.⁶¹⁻⁶⁴ CD40 is expressed on antigen presenting cells,⁶⁵ SCs, and osteoblasts.⁶⁶ CD40–CD40L is crucial for T cell activation and several functions of the immune system. It promotes macrophage activation and differentiation, antibody isotype switching, and the adequate organization of immunologic memory in B cells.⁶⁷ Binding to the integrins $\alpha_{IIb}\beta_3$,^{61, 62} Mac-1,⁶³ and $\alpha_5\beta_1$, which are widely expressed in the BM, is known to account for additional inflammatory effects of CD40L⁶⁴.

CD40L has been linked to post-natal skeletal maturation because children affected by X-linked hyper-IgM syndrome—a condition in which CD40L production is impaired due to a mutation of the CD40L gene—have low bone density.⁶⁸ Two mechanisms have been identified to link the CD40L–CD40 to skeletal maturation. First, activation of CD40 signaling in B cells by T cell expressed CD40L promotes production of the anti-osteoclastogenic factor OPG by B cells,¹⁶ thereby decreasing bone resorption. In addition, activation of CD40 signaling in SCs by T cell–expressed CD40L provides proliferative and survival cues to SCs both *in vitro* and *in vivo*.^{58, 66} CD40L also increases the commitment of SCs to the osteoblastic lineage.^{58, 69} At the same time, activation of CD40 signaling in osteoblastic cells increases their osteoclastogenic activity,^{58, 69} which is defined as the capacity of osteoblastic cells to induce and support osteoclast formation *in vitro*. Attesting to its relevance, CD40L is required for ovariectomy and continuous PTH treatment—a model of primary hyperparathyroidism—to expand SCs and their osteoblastic progeny and to increase the osteoclastogenic activity of SCs^{18, 58}. Underscoring the complexity of this regulatory system, T cells also suppress osteoclast formation by inducing CD80/86 signaling in osteoclast precursors by inducing the IDO/tryptophan pathway⁸.

Skeletal effects of PTH

Parathyroid hormone is an important endocrine regulator of calcium and phosphorus concentrations in extracellular fluid. Physiologic levels of circulating PTH are essential for maintaining serum and urinary calcium levels within their normal range. Chronic excessive PTH production is a cause of skeletal and extra skeletal disease. Secondary hyperparathyroidism has been implicated in the pathogenesis of senile osteoporosis,⁷⁰ while primary hyperparathyroidism (PHPT), is associated with accelerated bone loss,⁷¹ osteopenia,⁷²⁻⁷⁴ and increased bone turnover,⁷³ an independent risk factor for fractures. Furthermore, PHPT is a cause of extra-skeletal manifestations stemming from increased bone resorption such as hypercalcemia, recurrent nephrolithiasis, renal failure, peptic ulcers and mental changes.⁷² Primary and secondary hyperparathyroidism are mimicked by continuous PTH (cPTH) infusion. However when injected daily, a regimen known as

intermittent PTH (iPTH) treatment, the hormone markedly stimulates trabecular and cortical bone formation. Although this bone forming activity is antagonized, in part, by a stimulation of bone resorption, the net effect of iPTH is an improvement in bone microarchitecture and increased strength.⁷⁵⁻⁷⁷ As a result, intermittent treatment with the 1–34 fragment of PTH decreases the risk of fractures in humans, and is a U.S. Food and Drug Administration approved treatment modality for postmenopausal osteoporosis.^{78, 79} iPTH treatment is also under investigation as an agent for accelerating fracture repair and diminish the risk of non-unions.

Both cPTH and iPTH increase bone turnover in trabecular and cortical bone, as evidenced by elevations in histomorphometric and biochemical markers of resorption and formation,⁸⁰⁻⁸² whereas PHPT and cPTH treatment cause cortical bone loss by enhancing endosteal resorption through stimulation of OC formation, activity and life span.^{72, 82, 83} Severe chronic elevations of PTH levels may also lead to trabecular bone loss,^{72, 82} although PHPT and cPTH treatment often induce a modest increase in cancellous bone.^{73, 74, 80, 84} In contrast, iPTH treatment markedly increases trabecular bone volume due to a preponderant stimulation of trabecular bone formation, and causes a small loss of cortical bone.^{75, 78} PTH promotes bone formation by increasing the number of osteoblasts⁸⁵⁻⁸⁷ through multiple effects, including activation of quiescent lining cells,⁸⁸ increased OB proliferation^{89, 90} and differentiation,^{89, 91, 92} and attenuation of pre-OB and OB apoptosis.⁹³⁻⁹⁶

PTH exerts its biological activities by binding to the PPR receptor (also known as PTH-1R), which is expressed on SCs, osteoblasts and osteocytes⁹⁷⁻⁹⁹ but also T cells¹³ and macrophages.¹⁰⁰ SCs and osteoblasts were the first targets of PTH to be identified and earlier consensus developed that the catabolic effect of cPTH is mostly mediated by enhanced production of RANKL and decreased production of OPG by SCs and osteoblasts.^{76, 101, 102} More recent studies in mice with deletion and/or overexpression of PPR and RANKL in osteocytes^{32, 33, 99, 103} lead to the recognition that osteocytes represent essential targets of PTH in bone, and that increased production of RANKL by osteocytes plays an important role in cPTH induced bone loss.^{32, 33} However, some reports have ascribed a key role to OB produced RANKL.¹⁰⁴ osteocytes have also emerged as a critical target for the anabolic effects of PTH because transgenic mice expressing a constitutively active PTH receptor exclusively in osteocytes exhibit increased bone mass and bone remodeling, as well as reduced expression of the osteocyte-derived Wnt antagonist sclerostin, increased Wnt signaling, and decreased OB apoptosis.¹⁰⁵ Direct regulation of sclerostin production by osteocytes is regarded as a particularly relevant role in the anabolic activity of PTH.¹⁰⁵

One of the major effects of PTH is to activate Wnt signaling in all osteoblastic cells, including osteocytes. Activation of Wnt signaling induces OB proliferation¹⁰⁶ and differentiation,¹⁰⁷ prevents pre-OB and OB apoptosis,⁹⁵ and augments OB production of OPG.¹⁰⁸ Wnt proteins initiate a canonical signaling cascade by binding to receptors of the Frizzled family together with coreceptors, members of the low-density lipoprotein receptor-related protein (LRP) family, LRP5 and LRP6, which results in the stabilization of cytosolic β -catenin. A nuclear complex of β -catenin and the T cell factor/lymphoid enhancer factor (TCF/LEF) family of transcription factors then interacts with DNA to regulate the

transcription of Wnt target genes.¹⁰⁹ PTH is a canonical Wnt signaling agonist that increases β -catenin levels in osteoblastic cells.¹¹⁰ PTH, once bound to PPR, is also capable of forming a complex with LRP6 which results in LRP6 signaling and β -catenin activation.¹¹¹ Thus, PTH activates Wnt signaling in osteoblastic cells through both Wnt ligand-dependent and Wnt ligand-independent mechanisms.

The capacity of PTH to suppress the production of sclerostin,¹¹²⁻¹¹⁴ the finding that serum levels of sclerostin are inversely correlated with PTH levels in healthy women,¹¹⁵ and reports that women treated with teriparatide have decreased serum levels of sclerostin¹¹⁶ have led to the hypothesis that repression of the sclerostin gene (*SOST*) and the resulting inhibition of sclerostin production are a key mechanism of action of iPTH.¹¹⁷ Studies in *Sost* transgenic and global *Sost*^{-/-} mice revealed that sclerostin plays an important, yet non-exclusive, role in iPTH-induced anabolism.¹¹⁸⁻¹²⁰ The existence of a sclerostin-independent effect of iPTH was supported by the finding that iPTH equally elevates bone formation and resorption in WT and in *Sost* transgenic mice.¹¹⁸ In addition, iPTH induced a significant increase in trabecular thickness and mineral apposition rate in *Sost*^{-/-} mice, thus demonstrating that iPTH stimulates bone formation also independently of sclerostin suppression.¹¹⁸ Initial studies could not provide an answer to the question of whether iPTH increases trabecular bone volume in the absence of sclerostin, as no increase in bone mass was detected in the distal femur of both growing WT control and *Sost*^{-/-} mice in one study,¹¹⁸ while full anabolic activity of iPTH in the trabecular bone of skeletally-mature *Sost*^{-/-} mice was reported in another.¹²⁰ Uncertainty concerning the relevance of sclerostin-independent modalities of action of iPTH is the confounding effect of the altered baseline bone density that is characteristic of mice either lacking or overexpressing sclerostin.¹¹⁸⁻¹²⁰ The fact that iPTH blunts but does not completely block sclerostin production further limits the usefulness of *Sost*^{-/-} mice as a tool to investigate the mechanism of action of iPTH

PTH also regulates Dickkopf-1, a soluble LRP5 and LRP6 signaling inhibitor,¹¹⁰ and Sfrp-4, a factor that binds Wnt proteins and thereby antagonizes both canonical and non-canonical Wnt signaling.¹²¹ Uncertainty remains with regard to the identity and the source of Wnt ligands that activate Wnt signaling in response to PTH treatment.

BM macrophages are additional lineages involved in the mechanism of action of PTH. Depletion of macrophage precursors results in osteopenia and blunted iPTH anabolic activity.¹⁰⁰ By contrast, depletion of mature phagocytic macrophages potentiates iPTH-dependent anabolism by activating efferocytosis, a process that stimulates other BM cells to secrete Wn10b, Wnt3a, and TGF- β .¹⁰⁰ Moreover, iPTH stimulates macrophages to secrete TGF- β through an indirect effect mediated by sIL-6R, which is secreted by osteoblasts in response to iPTH.¹²² Although macrophages express the PTH receptor PPR,¹⁰⁰ the available data suggest that BMMs play a permissive role in the anabolic activity of iPTH. Another lineage known to express PPR are dendritic cells (DCs).¹²³ However, DCs have not been implicated in the mechanism of action of PTH in bone.

Role of T cells in primary hyperparathyroidism and the catabolic activity of continuous PTH treatment

T lymphocytes express functional PPR,¹³ respond to PTH,¹²⁴ and stimulate OB differentiation¹²⁵. These observations prompted more in depth investigations on the role of T cells as mediators of the pro-resorptive effect of cPTH treatment. These studies revealed that infusion of cPTH that mimics hyperparathyroidism fails to induce OC formation, bone resorption and cortical bone loss in mice lacking T cells.⁵⁸ By contrast, cPTH equally stimulated bone formation in T cell-replete and T cell-deficient mice. Interestingly, transplantation of tumors producing PTH and/or PTHrP in nude mice, a strain of T cell-deficient mice, stimulates bone resorption in spite of the absence of T cells in the host.¹²⁶⁻¹²⁸ The apparent discrepancy between these reports and those in which mice were treated with cPTH is explained by the higher levels of circulating PTH/PTHrP attained by transplanting PTH/PTHrP-producing tumors, as compared to those obtained by infusing cPTH at 80. Adding further support to this hypothesis are reports demonstrating that transplantation of adenomatous and hyperplastic parathyroid tissues in nude mice, in a fashion that elevates serum PTH to 80–240 ng/L, fails to induce hypercalcemia,^{129, 130} while parathyroid transplants that elevate serum PTH to ~1000 ng/L induce hypercalcemia in nude mice.¹³¹

T cells exert complex effects that are relevant for the effects of PTH in bone. The most upstream effect of cPTH is that of stimulating the production of TNF- α by both CD4⁺ and CD8⁺ T cells.¹³² Since CD8⁺ cells are more abundant in the BM than CD4⁺ cells, most of the TNF- α produced in the BM in response to cPTH originates from CD8⁺ cells.¹³² Attesting to the relevance of T cell produced TNF- α , cPTH fails to induce bone loss and stimulate bone resorption in mice specifically lacking T cell TNF- α production.¹³² PTH induces T cell production of TNF- α via direct activation of PPR signaling in T cells.¹³² Conditional silencing of the PTH receptor PPR in T cells blunts the stimulation of bone resorption induced by cPTH without affecting bone formation, thus blocking cortical bone loss and converting the effects of cPTH in trabecular bone from catabolic to anabolic.¹³² These findings demonstrate the critical relevance of direct PPR signaling in T cells. TNF- α stimulates osteoclast formation and activity via multiple mechanisms, which include increased production of RANKL by all osteoblastic cells including osteocytes. An important early effect of TNF- α is that of upregulating the expression of CD40 in SCs and osteoblasts, which increase their response to T cell expressed CD40L. Activation of CD40 signaling in osteoblastic cells increases the sensitivity of SCs and osteoblasts to cPTH, leading to a smaller suppression of OPG secretion in response to PTH and a resulting increase in the RANKL/OPG ratio. It should be noted that cPTH stimulates bone cells and immune cells to release growth factors and cytokines. Among them are TGF- β , IL-6, and TNF- α .^{122, 132-134} TGF- β and IL-6 direct the differentiation of naive CD4⁺ cells into T_H17 cells.^{26, 135, 136} Studies with agents neutralizing TNF- α have implicated TNF- α in the generation of T_H17 cells in rodents and humans.¹³⁷⁻¹³⁹ Recent data indeed suggest that cPTH-induce T_H17 cell differentiation, and that IL-17A produced by T_H17 cells, may act as an upstream cytokine that plays a pivotal role in the bone loss induced by cPTH and primary

hyperparathyroidism.³⁰ A fertile area of future investigation will be to determine the role of IL-17 in the bone loss caused by cPTH and PHPT.

Role of T cells in the anabolic activity of intermittent PTH treatment

Studies conducted in T cell deficient mice revealed that mice lacking T cells exhibit a blunted increase in bone formation and trabecular bone volume in response to iPTH¹³.

The importance of T cells for the bone anabolic activity of iPTH is shown in Figure 1, which depicts longitudinal sections of the femurs from WT and TCR β -deficient (*Tcrb*^{-/-}) mice treated with vehicle and iPTH. *Tcrb*^{-/-} mice are characterized by a complete deficiency of $\alpha\beta$ T cells. iPTH treatment for 4 weeks leads to the appearance of cuboidal lining osteoblasts in WT but not in *Tcrb*^{-/-} mice. Since cuboidal osteoblasts are actively engaged in bone formation, the images suggest that iPTH is not able to increase bone formation in *Tcrb*^{-/-} mice.

Furthermore, adoptive transfer of T cells into T cell deficient mice restored a normal bone anabolic response to iPTH. T cells augment the capacity of iPTH to improve architecture in trabecular but not in cortical bone. Although the reason of this selectivity is unknown, a lack of access of T cells to cortical surfaces is not a likely explanation, as T cells reach endosteal and periosteal bone surface through blood vessels and recirculate in and out of the BM.¹⁴⁰ With regard to the mechanism by which T cells potentiate the bone anabolic activity of iPTH, it is now clear that in the absence of T cells, iPTH is unable to increase the commitment of SCs to the osteoblastic lineage, induce OB proliferation and differentiation, and mitigate OB apoptosis. All of these actions of PTH were found to hinge on the capacity of T cells to activate Wnt signaling in osteoblastic cells.¹³ Although it is well established that Wnt activation is a key mechanism by which iPTH expands the osteoblastic pool, little information is available on the nature and the source of the Wnt ligand required to activate Wnt signaling in SCs and osteoblasts. BM CD8⁺ T cells produce large amounts of the osteogenic Wnt ligand Wnt10b.¹³ The relevance of CD8⁺ cells was demonstrated by the inability of iPTH to promote bone anabolism in major histocompatibility complex (MHC) class I-deficient mice, a strain that lacks CD8⁺ cells.¹³ Additional studies revealed that iPTH does not improve bone architecture in T cell-deficient mice reconstituted with CD4⁺ cells, while it does so in mice adoptively transferred with CD8⁺ cells.¹³ The pivotal role of T cell produced Wnt10b was revealed by the hampered effect of iPTH on bone volume in global *Wnt10b*^{-/-} mice¹⁴¹ and T cell-deficient mice reconstituted with T cells from *Wnt10b*^{-/-} mice.¹³ Together, the data indicate that CD8⁺ T cells potentiates the anabolic activity of PTH by providing Wnt10b, which is a critical Wnt ligand required for activating Wnt signaling in osteoblastic cells. Therefore, in the absence of CD8⁺ cells, stimulation of osteoblastic cells by PTH is not sufficient to elicit maximal Wnt activation due to the lack of a critical Wnt ligand.

Importantly, a recent report in human has shown that treatment with teriparatide, a form of iPTH treatment, increases the BM levels of Wnt10b.¹⁴² That study has also shown that T cells are the main source of Wnt10b in humans treated with teriparatide.¹⁴² By contrast,

patients affected by in primary hyperparathyroidism do not exhibit increased Wnt10b expression.¹⁴²

Additional interactions between T cells and SCs may contribute to the anabolic activity of iPTH. An important mediator of T cell–SC interaction is the T cell costimulatory ligand CD40L.¹⁴³ Attesting to the relevance of CD40L *in vivo*, silencing of CD40L blocks the effects of iPTH on SC proliferation, differentiation, and life span, resulting in blunted anabolic activity of iPTH on trabecular bone.

Together, the data suggest a requirement for a dual regulatory interaction between T cells and SCs. According to this model, silencing of either Wnt10b or CD40L is sufficient to blunt the responsiveness of SCs and their osteoblastic progeny to iPTH.

The residual bone anabolic activity of PTH observed in T cell–deficient mice is presumably due to ligand independent activation of LRP6,¹¹¹ and suppressed production of sclerostin.^{105, 112, 113} Indeed, it has recently been reported that mice treated with anti-sclerostin antibody maintain a partial anabolic response to iPTH. The sclerostin-independent activity of iPTH has shown to be completely due to increased production of Wnt10b by T cells.¹⁴⁴

In summary, the available data are consistent with a complex modality of action of iPTH that include suppression of sclerostin production and increased T cell production of Wnt10b (Fig. 2). In conditions of normal baseline bone turnover and bone mass and partial sclerostin blockade that mimic the repressive activity of the hormone on sclerostin production, iPTH stimulates osteoblastogenesis, OB life span, bone density, and trabecular bone volume independently of sclerostin through a Wnt10b-mediated mechanism.

Osteoblasts, osteocytes, and T cells are key targets of PTH

As discussed above, robust data have been published by many laboratories showing that silencing of PPR signaling in osteoblasts, osteocytes, or T cells results in the partial or total blockade of the bone anabolic and catabolic effects of PTH. These findings may sound surprising and perhaps contradictory if one envisions PTH acting using a parallel circuit regulatory modality. In this model, PTH targets osteoblasts, osteocytes, and T cells independently, and interactions between immune cells and bone cells do not play a significant role. This model forces investigators to focus on the effects of PTH on one cell population, for example on the capacity of the hormone to stimulate the osteocytic production of RANKL, and to underestimate the contributions of the effects of PTH on another lineage, for example the capacity to induce T cell production of TNF- α .

However, the fact that silencing of PPR signaling in one lineage (e.g., T cells) induces the same effects as silencing of PPR signaling in another (e.g., osteocytes) is in keeping with a serial circuit regulatory mode (Fig. 3). This model finds it justification on the fact that BM T cells provide cell surface signals and secrete cytokines that direct the differentiation of SCs toward osteoblasts characterized by a high sensitivity to PTH. Conversely, SCs that differentiate in a microenvironment devoid of T cells acquire a permanent low sensitivity to PTH, which is transmitted to their osteoblastic progeny (Fig. 4). Since osteoblasts

differentiate into osteocytes, low responsive osteocyte will arise from low responsive osteoblasts. Thus, by acting on SCs and early OB precursors, T cells have the capacity to set the responsiveness of all osteoblastic cells to PTH, including osteocytes. In addition, cytokines secreted by T cells, such as TNF- α and IL-17, target mature osteoblasts and osteocytes, regulating their production of RANKL.³⁰ We thus hypothesize the existence of a serial, rather than parallel, connection between T cells, osteoblasts, and osteocytes, with reciprocal, bidirectional regulatory interactions. T cells are upstream targets of PTH, while osteoblasts and osteocytes are downstream targets. Therefore, T cells, osteoblasts, and osteocytes are all critical targets of PTH.

Conclusions

An impressive amount of work published in the last 10 years has led to the recognition that T cells play an unexpected role in the regulation of bone resorption and bone formation through a variety of mechanisms. Studies in humans have corroborated observations in mice and shown the relevance of T cells for bone health in humans. Most of the evidence in humans has accrued in studies on the pathogenesis of postmenopausal osteoporosis. For example, evidence begins to emerge in favor of a role of T cell-produced TNF- α in postmenopausal bone loss in women,^{9, 10} and that in humans estrogen deficiency expands RANKL-expressing T and B cells.^{145, 146} Moreover, a role for IL-1 and TNF- α in humans is supported by reports that menopause increases the levels of these factors,¹⁴⁷⁻¹⁵¹ while treatment with inhibitors of IL-1 and TNF- α prevents the increase in bone resorption induced by estrogen deficiency.¹⁵²

Evidence has also emerged that T cells may contribute to the bone anabolic activity of teriparatide treatment in humans. These observations represent the first attempt to translate in humans the large body of evidence pointing to a role of T cells in the mechanism of action of PTH in the mouse. Preliminary reports show that hyperparathyroidism may elevate IL-17 production in humans, suggesting that human confirmation may be found for the studies on cPTH and T cells conducted in the mouse.

The most intriguing aspect of PTH biology remains the opposite response of the skeleton to continuous, versus intermittent, exposure to PTH. Several hypotheses have been proposed to explain the divergent response to cPTH and iPTH. Among them is a differential effect of cPTH and iPTH on OB life span, an hypothesis based on the observation that iPTH attenuates OB apoptosis⁹³⁻⁹⁶ while cPTH does not.⁹⁴ However, this explanation does not account for the differences in bone resorption elicited by iPTH and cPTH. *In vitro* studies have led to the alternative hypothesis that the bone response to PTH reflects the intermittent or continuous activation of PPR in bone cells.^{153, 154} However, this hypothesis does not explain why transgenic mice expressing a constitutively active PPR in osteoblasts or osteocytes exhibit a dramatic increase in trabecular bone formation^{97, 105} that resembles trabecular bone formation induced by iPTH. The capacity of T cells to secrete TNF- α and Wnt10 in response to cPTH and iPTH introduces a novel layer of complexity, but also offers new opportunities to fully understand the mechanism of action of PTH in bone.

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Figure 1.

Trichrome stained longitudinal sections of femurs from WT and *Tcrb*^{-/-} mice treated with daily injections of vehicle or human PTH (80 microgam/Kg/day) for 4 weeks. Lining osteoblasts acquire the typical morphology of bone forming osteoblasts (cuboidal appearance) in WT mice but not in *Tcrb*^{-/-} mice. A representative image is shown.

Figure 2.

Schematic representation of the mechanism of action of iPTH. In baseline conditions, T cells produce low levels of Wnt10b, whereas osteocytes secrete high levels of sclerostin. The presence of a low Wnt10b/sclerostin ratio prevents the activation of Wnt signaling in osteoblasts, leading to low osteoblastogenesis and high OB apoptosis. Treatment with iPTH increases the T cell production of Wnt10b and blunts the osteocytic secretion of sclerostin, thus increasing the Wnt10b/sclerostin ratio. Wnt10b is then capable of activating Wnt signaling in osteoblasts, leading to increased osteoblastogenesis and decreased OB apoptosis. Reproduced with permission from Ref. 144.

A **B** **Figure 3.**

Diagrammatic representation of published data demonstrating that silencing of PPR signaling in either T cells, osteoblasts, or osteocytes leads to either decreased or absent responses to cPTH and iPTH.^{32, 33, 99, 103, 132, 155} This type of response is consistent with a serial circuit regulatory mechanism.

Figure 4.

T cells lead to the differentiation of stromal cells (SCs) into PTH responsive osteoblasts (osteoblasts) and osteocytes (osteocytes). CD40L, a surface molecules expressed on T cells activates CD40 signaling in SCs, that selects and expands a population of SCs highly responsive to PTH. As SCs differentiate into osteoblasts and osteocytes, T cell-derived signaling conditions the entire osteoblastic progeny. (A) SCs from T cell-replete mice differentiate into osteoblasts and osteocytes highly sensitive to PTH. (B) SCs, osteoblasts, and osteocytes from T cell-deficient mice exhibit low sensitivity to PTH.