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The Role of T Lymphocytes in Bone Remodeling

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Abstract

Bone remodeling is a tightly regulated resorption and formation of bone matrix for physiological processes or to maintain bone function. Bone remodeling involves the synchronized differentiation and activity of bone-related cell types including bone matrix-depositing osteoblasts, bone matrix-resorbing osteoclasts, collagen/extracellular matrix-producing chondrocytes, and the progenitors of these cell types. T and B cells are adaptive immune cells that can influence bone remodeling by directly regulating the function of bone-related cells under normal and pathophysiological conditions. The specific mechanisms through which T cells control remodeling are not well defined. Here, we review the impact and influence of T cells and their products on the differentiation and function of bone cells during bone remodeling. Synthesizing new connections and highlighting potential mechanisms may promote additional avenues of study to elucidate the full role that immune cells play in regulating bone homeostasis.

Keywords

Osteoimmunology, Bone Homeostasis, Fracture, T Cells, B Cells, Osteoblasts, Osteoclasts, Chondrocytes

1. Bone Remodeling

Bone remodeling is an important component of bone homeostasis and the later stages of bone repair. It functions to protect the structural integrity of the skeletal system and contributes to the biochemical maintenance of the body's calcium and phosphorous levels. During this process old or damaged bone is resorbed and replaced with new bone material which occurs in three phases: bone matrix resorption, non-mineralized osteoid deposition, and osteoid maturation [1] [2].

During the resorption phase, monocyte/macrophage-derived osteoclasts secrete hydrogen ions and proteases to respectively, dissolve and destroy the min-

eral and proteinaceous components of bone matrix. Bone matrix resorption occurs at discrete locations on the bone to remove damaged bone tissue in advance of bone matrix deposition [1] [3]. Bone matrix resorption can also be stimulated by physiological cues, such as low serum calcium or the release of various mediators. For instance, T cells, B cells, or osteoblasts can secrete RANKL (Receptor activator of NF-$\kappa\beta$ Ligand) which can promote monocytes to differentiate into osteoclasts. RANKL is an essential cytokine that binds to RANK (Receptor activator of NF-$\kappa\beta$) receptor expressed on osteoclast progenitor cells resulting in the expression of osteoclast-related genes and acquisition of osteoclast phenotype [TRAP, Tartrate-resistant acid phosphatase activity, Cathepsin K gene expression, and bone matrix resorption] [3] [4]. RANKL is expressed by mature osteoblast cells, as well as T cells that have been activated under inflammatory conditions [4] [5] [6] [7]. Lymphocyte-associated RANKL expression is observed in the context of bone repair or diseases like rheumatoid arthritis [RA] or multiple myeloma [4] [8] [9].

During osteoid formation, mesenchymal stromal cells and other stem cell populations can be induced to differentiate into osteoblasts by growth factors, especially bone morphogenic proteins (BMPs) [1] [3] [10]. Osteoblasts secrete mainly Type 1 collagen (col1a1), lesser amounts of other collagens, and other extra-cellular matrix proteins to form non-mineralized osteoid. To mature the osteoid into bone matrix, enzymes secreted by osteoblasts, such as alkaline phosphatase, help catalyze the precipitation of calcium and the formation of hydroxyapatite, which is the primary mineral component of bone [11]. The hydroxyapatite crystalizes around the collagen fibers of the osteoid to produce mineralized and mature bone matrix [1] [3]. During this phase, osteoblasts may become trapped in the matrix and differentiate into specialized osteoblasts called osteocytes. The osteocytes sense mechanical load and help regulate bone mineral content and quality [12] [13] [14]. Together osteoblasts, osteoclasts, and osteocytes work in a coordinated manner to ensure bone remodeling is properly regulated.

2. Immune Cell Connections to Bone Remodeling

Cells of both the innate (monocytes/macrophages, dendritic cells, granulocytes, natural killer (NK) cells) and adaptive (T cell, B cells) immune system contribute to bone remodeling [15] [16] [17] [18] [19]. Innate and adaptive immune cells interact with their environment through the secretion of cytokines [15] [17]. These cytokines modulate the behavior and function of immune cells in an autocrine and paracrine manner and can regulate the function of other cell types and tissues via similar signaling pathways [20] [21] [22]. Pattern or inflammation activated innate immune cells serve as a source of key inflammatory cytokines such as Interlukin-1 (IL-1), Interlukin-6 (IL-6), Interferons (IFNs), Tumor Necrosis Factor-α (TNF-α). Although homeostatic bone remodeling is not typically associated with the immune response, immune-cell secreted cytokines may

directly or indirectly regulate the activity of bone-related cells while supporting immune surveillance/function (e.g., phagocytosis of cellular debris following apoptosis/necrosis programmed cell death) [2] [16] [18] [19] [23]. Likewise, bone-related cells may also modulate the behavior and activity of immune cells in the context of bone remodeling [24]. As such, understanding the role of immune cells, specifically T cells in bone remodeling may help identify secondary functions of the immune system.

3. T Lymphocyte Physiology

T cells are a major component of the adaptive immune system and function to protect the body from infection by external pathogens or from malignancies which may develop from transformed, abnormal cells. All T cells express a unique receptor called the T cell receptor (TCR). A TCR is capable of recognizing an antigen that is displayed on another cell through is major histocompatibility complex receptors (MHCs) [25]. T cells are characterized by the expression of a TCR, T cells overall represent a highly diverse and varied group of cells with unique phenotypes and functional characteristics. At the highest level, T cells are characterized by their expression of the co-receptors CD4 or CD8 [25] [26]. CD4 T cells or helper T cells play a critical role in response to infection or cellular transformation. These cells secrete cytokines in the secondary lymphoid and/or peripheral tissues in response to antigen which influences the differentiation and polarization of innate immune cells like granulocytes and macrophages as well as the development and differentiation of adaptive immune cells like B cells and other T cells [14] [16] [17]. Helper T cells can be further segregated into subsets based on the profile of secreted factors, typically driven by master transcriptional regulators (TRs) like T-bet for T helper 1 (Th1) cells and GATA3 for Th2 cells [25] [26] [27] [28].

Th1 cells express and secrete inflammatory cytokines like IFNγ and TNFα that serve to promote and enhance acute immune responses through activation of macrophages and dendritic cells. Inflammatory cytokines also facilitate the activity of CD8 T cells and induce the generation of IgG class immunoglobulins, the primary antibodies associated with pathogen neutralization through opsonization and complement-mediated destruction. [25] [26] [27] [28]. Conversely, Th2 cells secrete cytokines and other factors that are involved in the resolution of inflammatory responses and/or the polarization of granulocytes and the production of IgE which are the primary cell type and immunoglobulin involved in the anti-parasitic immune response, respectively [25] [26] [27] [28]. Th17 cells are another major class of helper T cell with diverse functions that include pro-inflammatory and anti-inflammatory elements. Th17 cells are characterized by the expression of the master transcriptional regulator, RORγt, and the secretion of IL-17 family of cytokines as well as IL-23 [29] [30]. IL-17 has been shown to contribute to the differentiation of osteoblasts and osteoclasts *in vitro* and *in vivo* and dysregulation of the IL-17 axis is associated with rheumatological dis-

eases that affect bone [31] [32] [33] [34] [35].

CD8 or cytotoxic T cells are the primary cell type associated with adaptive, cell-mediated cytolytic activity [25] [26] [36]. CD8 T cells express cellular machinery that is capable of inducing pore formation cellular membranes along with cytotoxic granules containing proteolytic enzymes that induce apoptosis in target cells [20] [37]. CD8 T cells become activated and licensed when they are presented antigen via major histocompatibility complex (MHC) I expressed on professional antigen presenting cells, like dendritic cells and macrophages [25] [36]. Activated CD8 T cells then survey the peripheral tissues and will recognize and kill cells that present that same antigen in a CD4 help-dependent manner. In addition to their cytolytic activity, CD8 T cells also secrete inflammatory cytokines that promote the activity phagocytic cells that migrate to the site of infection to phagocytose apoptotic bodies and cellular debris [25] [36].

Finally, a unique population CD4+ T cell exists, called regulatory T cells. Under the control of the transcriptional regulator, Fox3P, regulatory T cells express increased amounts of the IL-2 receptor (CD25) and decreased levels of the IL-7 receptor, CD127 [38] [39]. Regulatory T cells exhibit effectors functions that are anti-inflammatory in nature mediated by the secretion of anti-inflammatory cytokines like IL-4, IL-10, and TGFβ coupled with sequestration of IL-2 which is a necessary cytokine for homeostatic maintenance of Th and CD8 T cells [38] [39]. Dysfunction of regulatory T cells has been demonstrated to play a role in several diseases associated with osteo-degradation and bone metabolism dysregulation [40].

3.1. T Lymphocyte-Osteoblast Interactions

Osteoblast differentiation and activity is tightly regulated during bone remodeling. T cells produce cytokine mediators such as IL-17, IL-6, TGF-beta, and IFNγ which have been correlated with osteoblast maturation [4] [35] [41]. IL-17 is expressed by Th17 cells and can promote osteoblast differentiation and modify osteoblast activity (**Figure 1**) [30] [32] [42]. MSCs and pre-osteoblast cell lines exposed to IL-17A or IL-17F produced significantly more alkaline phosphatase [AP] activity [37]. *In vitro*, IL-17 induces osteoprogenitor cell proliferation and promotes osteoblast differentiation in a BMP-dependent and independent manner [32] [33] [35] [37].

Activated T cells can induce osteoblast IL-6 expression. Data has shown that osteoblast IL-6 expression can be enhanced by the addition of exogenous IL-17 to T cell/osteoblast co-cultures [47]. Like IL-17, IL-6 expression is upregulated during the early inflammatory response after bone injury [48] [49] [50]. IL-6 is involved in the development and differentiation of IL-17-producing Th17 cells which may indicate a positive feedback loop for osteoblastogenesis modulated by the Th17 intermediate [33]. IL-6 also inhibits the differentiation and activity of regulatory T-cells which may impede osteoblast differentiation and function via TGFβ and IL-10 signaling [33] [48]. In fact, IL-6 null mice have decreased bone

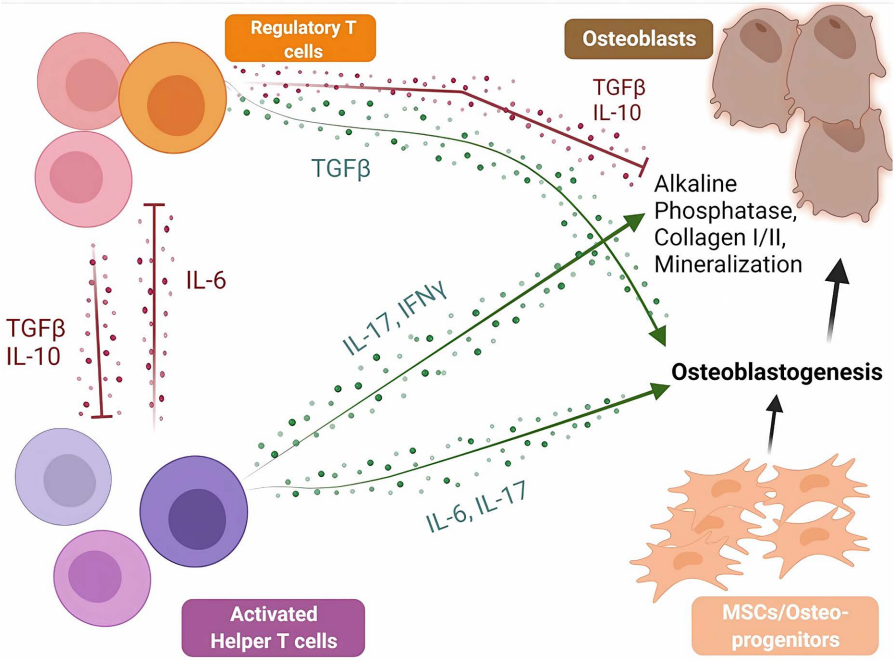

Figure 1. Multiple classes of T cells influence and regulate the behavior and activity of osteoblast in regarded to their differentiation and production of bone matrix depositing factors. Activated helper T cells (Th) secrete Inflammatory (IFNγ, IL-6) and immunomodulatory cytokines such as IL-17 that polarize mesenchymal stromal cells (MSCs) towards the osteoblast lineage and promote osteoblastogenesis. These same factors promote the expression of alkaline phosphatase (ALP), collagens I and II and increase mineralization activity of mature osteoblasts. Consequently, immunomodulatory T cells (Th2) or suppressive T cells (regulatory T cells) secrete factors such as TGFβ and IL-10 that globally inhibit the activity and function of activated T cells. This T cell-mediated suppression of T cell function is balanced by the activity of IL-6 which inhibits the differentiation of immuno-suppressive T cell subsets and promotes inflammatory conditions. In parallel, TGFβ and IL-10 can reduce bone matrix deposition by osteoblasts, while conversely, TGFβ alone may promote osteoblastogenesis in a localized manner [2] [32] [37] [40] [43] [44] [45] [46].

mineral density, callus crystallinity, and bone volume at early and intermediate points of bone remodeling post-fracture [49] [50]. This highlights the potential role of T-cell secreted IL-6 in osteoblast function [14] [50] [51] [52].

TGFβ is another immunoregulatory cytokine produced by regulatory T cells. It functions to promote resolution of inflammation by inhibiting the activity of innate and adaptive immune cells [10]. TGFβ also acts as an important factor in the differentiation of Th17 cells as well as bone remodeling [10] [29] [30]. *In vivo* knockdown of functional TGFβ receptors in MSC lineage cells resulted in increased trabecular stiffness and bone mineral density. Interestingly, these changes in strength appear to be a result of decreases in osteocyte densities rather than osteoblast-dependent responses [33] [51]. Furthermore, Joyce *et al.* observed a localized increase in osteoblasts in the subperiosteal area of rat femurs following continual injection of TGFβ independent of bone or cartilage injury. However, within the same study, exogenous TGFβ decreased the expression of bone asso-

ciated markers [collagen 1a and collagen 2] [52]. These opposing results may suggest that modulation of osteoblast differentiation by TGFβ may be dependent on secondary or tertiary signals produced by other cells (possibly T cells) during the remodeling process. Further studies in this area are necessary to define the full context of TGFβ and its diametrically opposed impact on osteoblast differentiation and function. More specifically, future studies could determine which cell types and cytokines modulate TGFβ expression and influence osteoblast recruitment and function during normal bone remodeling.

Another T cell secreted cytokine known to stimulate osteoblast differentiation and activity is IFNγ [4] [37]. Activated CD4+ Th1, CD8+ cytotoxic T cells, and innate natural killer lymphocytes are a significant source of IFNγ [4] [53] [54]. IFNγ is an important early autocrine and paracrine factor that promotes pro-inflammatory responses of innate and adaptive immune cells and is expressed/secreted early-on in the inflammatory response to infection or injury [21]. Autocrine secreted IFNγ by osteoblast committed MSCs has been observed to promote early osteoclastogenic commitment while the addition of exogenous IFNγ further accelerated osteoblastogenesis [54]. Inhibition of IFNγ signaling, either through *in vivo* knock-out of the IFNγ receptor or *in vitro* blockade of IFNγ via neutralizing antibodies, demonstrated the ability to impede proliferation of osteoprogenitors and attenuate osteoblastogenesis [37] [54]. Combined, the literature demonstrates a connection between these T cell secreted mediators and components of the bone remodeling process which warrants further investigation.

3.2. T Lymphocyte-Osteoclast Interactions

Osteoclast activity is tightly regulated by external stimuli derived from accessory cell types during normal bone remodeling, injury or disease (**Figure 2**). Activation of the T cell receptor (TCR) promotes T cell RANKL expression. T cell expressed RANKL can be surface-bound or secreted [5] [6]. As previously discussed, IFNγ is upregulated in activated T cells and may be co-expressed with RANKL [55] [56]. IFNγ is an important cytokine in the modulation of monocyte/macrophage function. As osteoclasts are derived from monocytes, IFNγ produced from T cells may also regulate osteoclastogenesis. Specifically, IFNγ exposure decreases the response of osteoclasts to RANKL signaling by targeting TRAF6, the key second messenger in the RANK signaling pathway [56]. Furthermore, the ability of activated, IFNγ+ T cells to induce osteoclastogenesis *in vitro* is enhanced in the presence of IFNγ neutralizing antibodies in a dose-dependent manner [55]. These experimental results suggest that RANKL induction of osteoclastogenesis by T cells is mitigated during inflammation-related expression of IFNγ by T cells.

Through expression of co-stimulatory ligands such as CD40L, T cells provide important stimulatory and regulatory signals to B cells in primary and secondary lymphoid tissue [57] [58]. Meendnu *et al.* identified the presence of RANKL-

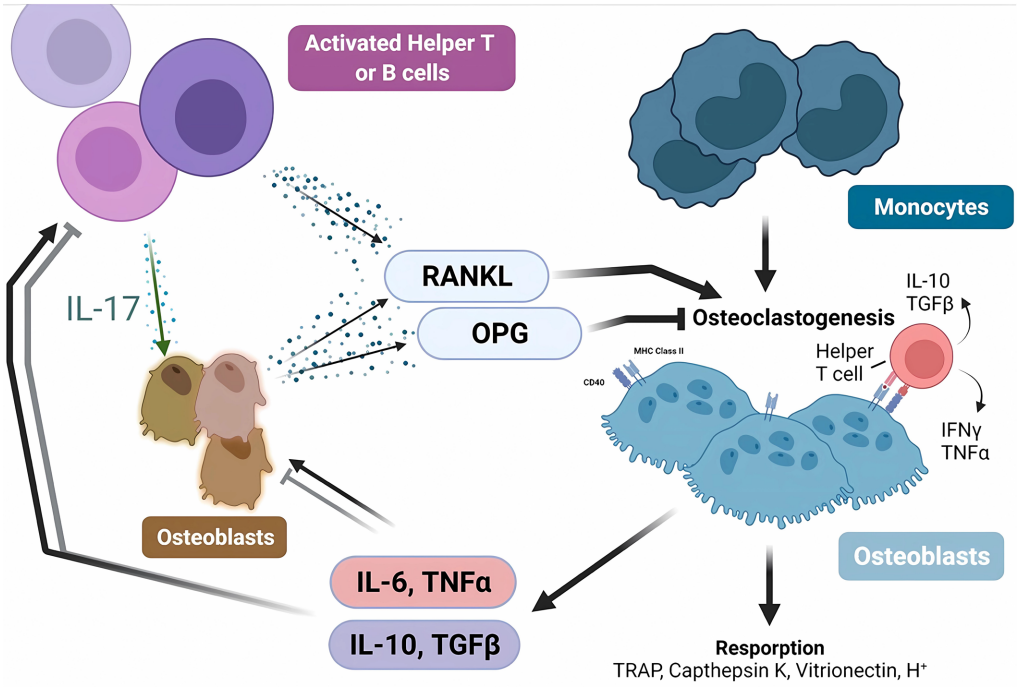

Figure 2. A general graphical summary of adaptive lymphocyte (T cell and B cell) interactions and cross-talk with osteoclasts and their progenitors (monocytes) in context of bone homeostasis. T cells and/or B cells that have been activated by inflammatory stimuli secrete RANKL which is a potent inducer of osteoclastogenesis. Additionally, Th17 Helper T cells secrete IL-17 that polarizes osteoprogenitors to differentiate into osteoblasts which also secrete receptor activator of nuclear factor Kappa-B ligand (RANKL). Functional osteoclasts produce factors that degrade both the mineral and proteinaceous components of bone. In parallel, osteoclasts interact with T cells in an MHC II-dependent manner leading to secretion of immunomodulatory cytokines that can alternatively drive or inhibit activation of T/B cells and either promote T cell and osteoblast-mediated secretion of RANKL or the expression of the RANKL decoy receptor, osteoprotegerin (OPG) from osteoblasts.

expressing B cells in the peripheral blood and synovial fluid of rheumatoid arthritis patients. RANKL expression was induced when CD40 ligation and PMA induced class-switching in memory B cells [CD27+IgD−] [9]. Similarly, Yoe *et al.* observed RANKL mRNA expression in B cells isolated from synovial fluid of RA patients with excess lymphocyte [CD4+ and CD8+ T cells] and myeloid [monocytes and neutrophils] [59]. These RANKL producing B cells may be modulated by macrophage and T cell expressed IFNγ which has been correlated with decreases in osteoprotegerin [OPG] expression [59]. OPG acts as a decoy receptor for RANKL, sequestering RANKL from binding to and activating RANK and RANK-associated signal transduction [60]. Concordantly, the RANK/OPG ratio is an important modulator of bone remodeling. Evaluation of RANK and OPG mRNA and immunoreactivity has temporally localized expression of each factor to the mineral resorption and deposition phases, respectively in a model of bone remodeling. Evaluation of age or HIV-related osteoporotic bone loss associates with dysregulation and shift of the RANKL/OPG axis towards the predominance of RANKL leading to increase osteoclast differentiation and resorption [60] [61] [62].

Estrogen deficiency is an established driver of osteoporotic bone degradation. The immune mechanisms that contribute to increased bone resorption because of estrogen loss are poorly understood [7] [63]. T cell activation is elevated in pre-menopausal women following ovariectomy resulting in increases in T cell-derived RANKL potentially contributing to osteoporosis [64]. However, some studies evaluating T cell responses in ovariectomized suggest no dysregulated activation [65] [66]. Specifically, ovariectomized mice developed normal bone homeostasis upon substitution with estradiol regardless of ERα expression on T cells [45].

Osteoclasts may influence the behavior and states of T cells in cell-cell contact dependent manner by modulating MHC expression. Osteoclasts express some of the same inflammatory mediators that are produced by professional antigen presenting cells [APCs] including key HLA genes [HLA-ABC, HLA-DR/DQ], costimulatory ligands [CD80, CD86], and co-stimulatory receptors including CD40 [67] [68]. Additionally, osteoclasts may produce an array of immunomodulatory cytokines such IL-10, IL-6 TNFα, TGFβ which modulates T cell responses in 2˚ lymphoid and peripheral tissues [67] [68]. For example, induction of a suppressive FoxP3+ CD8 T cells by self-antigen presenting osteoclasts resulting in osteoclast suppression is an example of this feedback loop [67]. While osteoclasts may not necessarily serve as primary antigen presenting cells in context of immune protection; the ability to interact with T cells via endogenous factors and induce specific behaviors in T cells may play a role in regulating osteoclast activity important to bone homeostasis.

3.3. T Lymphocyte-Chondrocyte Interactions

Chondrocytes are derived from MSCs and represent the primary extra cellular matrix [ECM] producing cells associated with bone and cartilage matrix. Mature chondrocytes maintain these tissues by producing new collagen and proteoglycans in response to environmental cues. Within standard physiological conditions, chondrocytes are shielded and protected from T and B cells as the ECM is generally devoid of immune cells. In context of this immune privilege, chondrocytes have been defined as potent immunomodulators with the ability to substantially influence the behavior and responses of T cells and macrophages (**Figure 3**). Research has shown that human articular chondrocytes [hACs] can inhibit activation-induced proliferation of allogeneic T cells in a contact-dependent manner [24] [69]. One potential mechanism suggests that chondrocytes downregulate IL-2 receptor expression on T cells which decreases T cell activation [70].

Another chondrocyte mediated mechanism involves modulation of prostaglandin E2 [PGE2] secretion to attenuate the differentiation of monocytes into mature antigen presenting macrophages and dendritic cells [24]. The loss of these cell types ultimately suppresses the activity of effector T cells which disrupt immune cell regulation of bone homeostasis [46] [71] [72] [73] [74]. Other research has shown that *in vitro* cultured-derived chondrocytes can be immunomodulatory

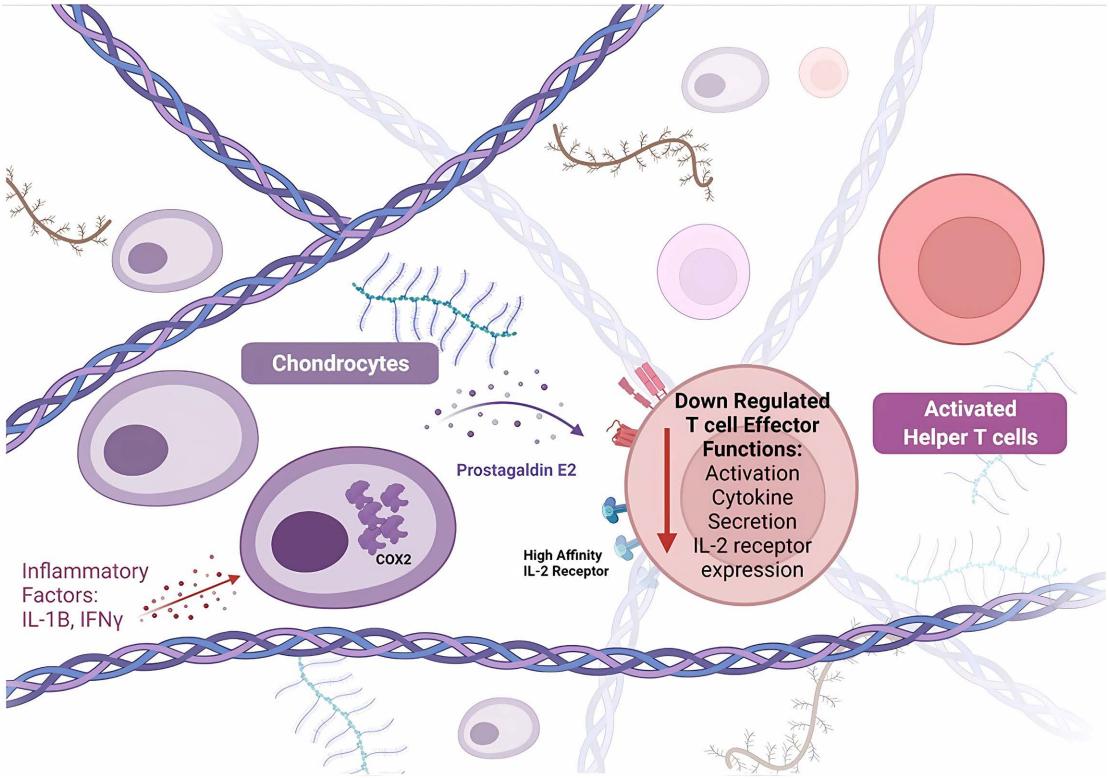

Figure 3. Modulation and suppression of immune cell activity by chondrocytes may protect the immune-privileged state of cartilage and serve as switch to suppress the acute inflammatory response associated with fracture injury and healing. Inflammatory stimuli caused by local injury induces the expression and activity of cyclooxygenase 2 (COX2) in chondrocytes, a key intermediate in the arachidonic acid cascade which promotes the production of prostaglandin E2 (PGE2). PGE2 is a potent immunomodulatory factor that suppresses the function of inflammatory T cells and promotes the differentiation of immunomodulatory T cells subsets and alternative polarized (M2) macrophages. Chondrocytes are most active during the intermediate phase of fracture healing responses during which they actively produce extra-cellular matrix proteins to stabilize the fracture hematoma as a soft callus. This coincides with the latter phases of the acute inflammatory response during which inflammatory T cells, B cells, and innate immune cells emigrate from the hematoma/callus resulting the development of a more anti-inflammatory, immunomodulatory, and angiogenic environment.

and inhibit antigen and non-antigen specific T cell proliferation and production of pro-cytolytic factors such as granzyme B [69] [75]. Despite these observations, the exact mechanisms by which chondrocytes exert immunomodulatory effects is not understood as these cells lack the machinery to regulate T cell functions such as MHC II and inhibitor cell surface ligands [69] [75].

Conversely, data from Nakamura *et al.* suggest a pro-inflammatory role for chondrocytes and their interactions with T cells in osteoarthritis [OA]. In this study, co-cultures of autologous chondrocytes and T cells from OA patients and health donors demonstrate an overall increase in T cell-expressed inflammatory mediators MMP-1, 3, 13 and the chemokine, RANTES [CCL5] [76]. These inflammatory mediators promote broad chemotactic effects important to both innate and adaptive immune cells [20] [76]. More specifically, upregulation of RANTES expression was found in OA derived chondrocytes and T cells that were associated with dysregulated bone remodeling [76].

4. Bone-Related Immune to Immune Cell Interactions

Bone matrix is a critically important site for the development and homeostasis of the immune system. Hematopoietic progenitors arise in the bone marrow and initially differentiate into erythroid, myeloid, and lymphoid progenitors which then go on to differentiate in red blood cells/platelets, macrophages/dendritic cell/granulocytes, and NK/B/T cells respectively. In their mature/differentiated states, these immune cells constantly migrate to and from the bone marrow and matrix. Some immune cells, such as osteo-macrophages, permanently reside within the bone matrix in proximity to bone metabolizing cells and directly influence their behavior [77] [78]. Dendritic cells are a specific sub-lineage of myeloid cell specialized in antigen presentation and licensing of adaptive lymphocytes. As myeloid-derived cells, dendritic cells share a common progenitor with osteoclasts. In specific scenarios, dendritic cells may trans-differentiate into bone resorbing osteoclasts; however, it is not yet established whether this process is homeostatic or associated with disease etiology [79]. As with osteoclasts, the RANK/RANKL axis contributes to regulation of dendritic cell activity. Specifically, via the interaction with T cell secreted TNF-related activation induced cytokine [TRANCE], RANK may induce an immunomodulatory or immunosuppressive phenotype marked by the production of immunomodulatory cytokines such as IL-10 and TGFβ [68] [80]. This immunomodulatory phenotype and function may be a component of a RANKL feedback loop that induces are more pro-osteogenic environment as both IL-10 and TGFβ promote differentiation of osteoblasts and inhibit chemotaxis, activation, and differentiation of monocytes [22] [43].

5. Conclusions

This review explores the basic principles and current understanding regarding the roles of T lymphocytes in regulating elements of bone remodeling. Specifically, the interactions of T lymphocytes were discussed in context the differentiation and function of osteoblasts, osteoclasts, and chondrocytes and their roles in bone remodeling. There is a growing body of evidence highlighting the role of immune cells in regulating bone remodeling independent of acute, injury-related inflammatory responses. T cells are present in all tissues and constantly surveil their local environment, including bone matrix, for foreign antigen and evidence of cellular transformation. Cell-to-cell contact and paracrine signaling is associated with this surveillance mechanism and serves as a potential route by which T cells interact with and modulate activity of bone-related cells.

Despite the expanding knowledge in the field of osteoimmunology, the core mechanisms by which immune cells regulate bone remodeling needs to be further studied. Critically, knowledge remains sparse in key areas that connect inflammatory pathways to regulation of bone metabolism. Expanding understanding by exploration to identify key immune cell networks and associated molecular pathways impacting the homeostatic process will help to develop bet-

ter therapeutics and treatment for the unmet medical need associated with done metabolism-related diseases. To fully leverage this potential, continued development and optimization of basic and disease-related experimental models is critical. This includes higher throughput *in vitro* and ex vivo models which may be able to replicate some of the physiological conditions of bone matrix formation, and the balance of its deposition and resorption as well as translational models of *in vivo* homeostasis and disease to bridge between mechanism and therapeutic intent/potential.

Conflicts of Interest

The authors attest that the work summarized herein was conducted in that absence of any financial or commercial influences that could be considered conflicts of interest.

Contributions

Review concept was conceived by JA Cottrell and JA Fuller. Literature searches, review, and primary authorship was conducted by JA Fuller, RE Samuel, JJ Abraham, D Gad, DM Padilla. Primary editing and critical review was conducted by JA Cottrell and JL Fuller. All authors read and approved the final draft.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

References

[1] Hadjidakis, D.J. and Androulakis, I.I. (2006) Bone Remodeling. *Annals of the New York Academy of Sciences*, **1092**, 385-396. https://doi.org/10.1196/annals.1365.035

[2] Ono, T. and Takayanagi, H. (2017) Osteoimmunology in Bone Fracture Healing. *Current Osteoporosis Reports*, **15**, 367-375. https://doi.org/10.1007/s11914-017-0381-0

[3] Xiao, W., *et al.* (2016) Cellular and Molecular Aspects of Bone Remodeling. *Frontiers of Oral Biology*, **18**, 9-16. https://doi.org/10.1159/000351895

[4] Konnecke, I., *et al.* (2014) T and B Cells Participate in Bone Repair by Infiltrating the Fracture Callus in a Two-Wave Fashion. *Bone*, **64**, 155-165. https://doi.org/10.1016/j.bone.2014.03.052

[5] Walsh, N.C., *et al.* (2013) Activated Human T Cells Express Alternative mRNA Transcripts Encoding a Secreted Form of RANKL. *Genes & Immunity*, **14**, 336-345. https://doi.org/10.1038/gene.2013.29

[6] Wang, R., *et al.* (2002) Regulation of Activation-Induced Receptor Activator of NF-kappaB Ligand (RANKL) Expression in T Cells. *European Journal of Immunology*, **32**, 1090-1098. https://doi.org/10.1002/1521-4141(200204)32:4<1090::AID-IMMU1090>3.0.CO;2-P

[7] Xiong, J., *et al.* (2018) Soluble RANKL Contributes to Osteoclast Formation in Adult Mice but Not Ovariectomy-Induced Bone Loss. *Nature Communications*, **9**, 2909. https://doi.org/10.1038/s41467-018-05244-y

[8] Giuliani, N., *et al.* (2002) Human Myeloma Cells Stimulate the Receptor Activator of Nuclear Factor-KappaB Ligand (RANKL) in T Lymphocytes: A Potential Role in Multiple Myeloma Bone Disease. *Blood*, **100**, 4615-4621. https://doi.org/10.1182/blood-2002-04-1121

[9] Meednu, N., *et al.* (2016) Production of RANKL by Memory B Cells: A Link between B Cells and Bone Erosion in Rheumatoid Arthritis. *Arthritis & Rheumatology*, **68**, 805-816. https://doi.org/10.1002/art.39489

[10] Chen, G., Deng, C. and Li, Y.P. (2012) TGF-Beta and BMP Signaling in Osteoblast Differentiation and Bone Formation. *International Journal of Biological Sciences*, **8**, 272-288. https://doi.org/10.7150/ijbs.2929

[11] Murshed, M. (2018) Mechanism of Bone Mineralization. *Cold Spring Harbor Perspectives in Medicine*, **8**, a031229. https://doi.org/10.1101/cshperspect.a031229

[12] Hoshi, K., *et al.* (2014) Compressive Force-Produced CCN2 Induces Osteocyte Apoptosis through ERK1/2 Pathway. *Journal of Bone and Mineral Research*, **29**, 1244-1257. https://doi.org/10.1002/jbmr.2115

[13] Komori, T. (2013) Functions of the Osteocyte Network in the Regulation of Bone Mass. *Cell and Tissue Research*, **352**, 191-198. https://doi.org/10.1007/s00441-012-1546-x

[14] McGregor, N.E., *et al.* (2019) IL-6 Exhibits both cis- and trans-Signaling in Osteocytes and Osteoblasts, but Only Trans-Signaling Promotes Bone Formation and Osteoclastogenesis. *Journal of Biological Chemistry*, **294**, 7850-7863. https://doi.org/10.1074/jbc.RA119.008074

[15] Bonilla, F.A. and Oettgen, H.C. (2010) Adaptive Immunity. *Journal of Allergy and Clinical Immunology*, **125**, S33-S40. https://doi.org/10.1016/j.jaci.2009.09.017

[16] Charles, J.F. and Nakamura, M.C. (2014) Bone and the Innate Immune System. *Current Osteoporosis Reports*, **12**, 1-8. https://doi.org/10.1007/s11914-014-0195-2

[17] Gasteiger, G., *et al.* (2017) Cellular Innate Immunity: An Old Game with New Players. *Journal of Innate Immunity*, **9**, 111-125. https://doi.org/10.1159/000453397

[18] Walsh, M.C., *et al.* (2018) Updating Osteoimmunology: Regulation of Bone Cells by Innate and Adaptive Immunity. *Nature Reviews Rheumatology*, **14**, 146-156. https://doi.org/10.1038/nrrheum.2017.213

[19] Warnke, P.H., *et al.* (2006) Innate Immunity in Human Bone. *Bone*, **38**, 400-408. https://doi.org/10.1016/j.bone.2005.09.003

[20] Dinarello, C.A. (2000) Proinflammatory Cytokines. *Chest*, **118**, 503-508. https://doi.org/10.1378/chest.118.2.503

[21] Gosain, A. and Gamelli, R.L. (2005) A Primer in Cytokines. *Journal of Burn Care & Research*, **26**, 7-12. https://doi.org/10.1097/01.BCR.0000150214.72984.44

[22] Opal, S.M. and DePalo, V.A. (2000) Anti-Inflammatory Cytokines. *Chest*, **117**, 1162-1172. https://doi.org/10.1378/chest.117.4.1162

[23] Schneider, P.S., Sandman, E. and Martineau, P.A. (2018) Osteoimmunology: Effects of Standard Orthopaedic Interventions on Inflammatory Response and Early Fracture Healing. *Journal of the American Academy of Orthopaedic Surgeons*, **26**, 343-352. https://doi.org/10.5435/JAAOS-D-16-00646

[24] Pereira, R.C., *et al.* (2016) Human Articular Chondrocytes Regulate Immune Response by Affecting Directly T Cell Proliferation and Indirectly Inhibiting Monocyte Differentiation to Professional Antigen-Presenting Cells. *Frontiers in Immunology*, **7**, 415. https://doi.org/10.3389/fimmu.2016.00415

[25] Pennock, N.D., *et al.* (2013) T Cell Responses: Naive to Memory and Everything in between. *Advances in Physiology Education*, **37**, 273-283. https://doi.org/10.1152/advan.00066.2013

[26] Germain, R.N. (2002) T-Cell Development and the CD4-CD8 Lineage Decision. *Nature Reviews Immunology*, **2**, 309-322. https://doi.org/10.1038/nri798

[27] Murphy, K.M. and Reiner, S.L. (2002) The Lineage Decisions of Helper T Cells. *Nature Reviews Immunology*, **2**, 933-944. https://doi.org/10.1038/nri954

[28] Saravia, J., Chapman, N.M. and Chi, H. (2019) Helper T Cell Differentiation. *Cellular & Molecular Immunology*, **16**, 634-643. https://doi.org/10.1038/s41423-019-0220-6

[29] Stockinger, B. (2007) Good for Goose, but Not for Gander: IL-2 Interferes with Th17 Differentiation. *Immunity*, **26**, 278-279. https://doi.org/10.1016/j.immuni.2007.03.001

[30] Stockinger, B. and Veldhoen, M. (2007) Differentiation and Function of Th17 T Cells. *Current Opinion in Immunology*, **19**, 281-286. https://doi.org/10.1016/j.coi.2007.04.005

[31] Le Goff, B., *et al.* (2019) Implication of IL-17 in Bone Loss and Structural Damage in Inflammatory Rheumatic Diseases. *Mediators of Inflammation*, **2019**, Article ID: 8659302. https://doi.org/10.1155/2019/8659302

[32] Liu, W.X., *et al.* (2015) The Role of T Helper 17 Cells and Other IL-17-Producing Cells in Bone Resorption and Remodeling. *International Reviews of Immunology*, **34**, 332-347. https://doi.org/10.3109/08830185.2014.952414

[33] Nam, D., *et al.* (2012) T-Lymphocytes Enable Osteoblast Maturation via IL-17F during the Early Phase of Fracture Repair. *PLOS ONE*, **7**, e40044. https://doi.org/10.1371/journal.pone.0040044

[34] Noonan, K., *et al.* (2010) A Novel Role of IL-17-Producing Lymphocytes in Mediating Lytic Bone Disease in Multiple Myeloma. *Blood*, **116**, 3554-3563. https://doi.org/10.1182/blood-2010-05-283895

[35] Ono, T., *et al.* (2016) IL-17-Producing Gamma-Delta T Cells Enhance Bone Regeneration. *Nature Communications*, **7**, 10928. https://doi.org/10.1038/ncomms10928

[36] Joshi, N.S. and Kaech, S.M. (2008) Effector CD8 T Cell Development: A Balancing Act between Memory Cell Potential and Terminal Differentiation. *The Journal of Immunology*, **180**, 1309-1315. https://doi.org/10.4049/jimmunol.180.3.1309

[37] Croes, M., *et al.* (2016) Proinflammatory T Cells and IL-17 Stimulate Osteoblast Differentiation. *Bone*, **84**, 262-270. https://doi.org/10.1016/j.bone.2016.01.010

[38] Hori, S. and Sakaguchi, S. (2004) Foxp3: A Critical Regulator of the Development and Function of Regulatory T Cells. *Microbes and Infection*, **6**, 745-751. https://doi.org/10.1016/j.micinf.2004.02.020

[39] Luo, C.T. and Li, M.O. (2013) Transcriptional Control of Regulatory T Cell Development and Function. *Trends in Immunology*, **34**, 531-539. https://doi.org/10.1016/j.it.2013.08.003

[40] Jiang, H., *et al.* (2018) Downregulation of Regulatory T Cell Function in Patients with Delayed Fracture Healing. *Clinical and Experimental Pharmacology and Physiology*, **45**, 430-436. https://doi.org/10.1111/1440-1681.12902

[41] Andrew, J.G., *et al.* (1994) Inflammatory Cells in Normal Human Fracture Healing. *Acta Orthopaedica Scandinavica*, **65**, 462-466. https://doi.org/10.3109/17453679408995493

[42] Huang, H., *et al.* (2009) IL-17 Stimulates the Proliferation and Differentiation of

Human Mesenchymal Stem Cells: Implications for Bone Remodeling. *Cell Death & Differentiation*, **16**, 1332-1343. https://doi.org/10.1038/cdd.2009.74

[43] Amarasekara, D.S., Kim, S. and Rho, J. (2021) Regulation of Osteoblast Differentiation by Cytokine Networks. *International Journal of Molecular Sciences*, **22**, 2851. https://doi.org/10.3390/ijms22062851

[44] El Khassawna, T., *et al.* (2017) T Lymphocytes Influence the Mineralization Process of Bone. *Frontiers in Immunology*, **8**, 562. https://doi.org/10.3389/fimmu.2017.00562

[45] Gustafsson, K.L., *et al.* (2018) ERalpha Expression in T Lymphocytes Is Dispensable for Estrogenic Effects in Bone. *Journal of Endocrinology*, **238**, 129-136. https://doi.org/10.1530/JOE-18-0183

[46] Li, M., *et al.* (2014) Mesenchymal Stem Cells Suppress CD8+ T Cell-Mediated Activation by Suppressing Natural Killer Group 2, Member D Protein Receptor Expression and Secretion of Prostaglandin E2, Indoleamine 2,3-Dioxygenase and Transforming Growth Factor-Beta. *Clinical and Experimental Immunology*, **178**, 516-524. https://doi.org/10.1111/cei.12423

[47] Stanley, K.T., *et al.* (2006) Immunocompetent Properties of Human Osteoblasts: Interactions with T Lymphocytes. *Journal of Bone and Mineral Research*, **21**, 29-36. https://doi.org/10.1359/JBMR.051004

[48] Prystaz, K., *et al.* (2018) Distinct Effects of IL-6 Classic and Trans-Signaling in Bone Fracture Healing. *The American Journal of Pathology*, **188**, 474-490. https://doi.org/10.1016/j.ajpath.2017.10.011

[49] Wallace, A., *et al.* (2011) Effects of Interleukin-6 Ablation on Fracture Healing in Mice. *Journal of Orthopaedic Research*, **29**, 1437-1442. https://doi.org/10.1002/jor.21367

[50] Yang, X., *et al.* (2007) Callus Mineralization and Maturation Are Delayed during Fracture Healing in Interleukin-6 Knockout Mice. *Bone*, **41**, 928-936. https://doi.org/10.1016/j.bone.2007.07.022

[51] Filvaroff, E., *et al.* (1999) Inhibition of TGF-beta Receptor Signaling in Osteoblasts Leads to Decreased Bone Remodeling and Increased Trabecular Bone Mass. *Development*, **126**, 4267-4279. https://doi.org/10.1242/dev.126.19.4267

[52] Joyce, M.E., *et al.* (1990) Role of Transforming Growth Factor-Beta in Fracture Repair. *Annals of the New York Academy of Sciences*, **593**, 107-123. https://doi.org/10.1111/j.1749-6632.1990.tb16104.x

[53] Castro, F., *et al.* (2018) Interferon-Gamma at the Crossroads of Tumor Immune Surveillance or Evasion. *Frontiers in Immunology*, **9**, 847. https://doi.org/10.3389/fimmu.2018.00847

[54] Duque, G., *et al.* (2009) Autocrine Regulation of Interferon Gamma in Mesenchymal Stem Cells Plays a Role in Early Osteoblastogenesis. *Stem Cells*, **27**, 550-558. https://doi.org/10.1634/stemcells.2008-0886

[55] Kotake, S., *et al.* (2005) IFN-Gamma-Producing Human T Cells Directly Induce Osteoclastogenesis from Human Monocytes via the Expression of RANKL. *European Journal of Immunology*, **35**, 3353-3363. https://doi.org/10.1002/eji.200526141

[56] Takayanagi, H., *et al.* (2000) T-Cell-Mediated Regulation of Osteoclastogenesis by Signalling Cross-Talk between RANKL and IFN-Gamma. *Nature*, **408**, 600-605. https://doi.org/10.1038/35046102

[57] Crotty, S. (2011) Follicular Helper CD4 T Cells (TFH). *Annual Review of Immunology*, **29**, 621-663. https://doi.org/10.1146/annurev-immunol-031210-101400

[58] Li, Y., *et al.* (2007) B Cells and T Cells Are Critical for the Preservation of Bone Homeostasis and Attainment of Peak Bone Mass *in Vivo*. *Blood*, **109**, 3839-3848. https://doi.org/10.1182/blood-2006-07-037994

[59] Ota, Y., *et al.* (2016) Generation Mechanism of RANKL(+) Effector Memory B Cells: Relevance to the Pathogenesis of Rheumatoid Arthritis. *Arthritis Research & Therapy*, **18**, 67. https://doi.org/10.1186/s13075-016-0957-6

[60] Tanaka, H., *et al.* (2011) Expression of RANKL/OPG during Bone Remodeling *in Vivo*. *Biochemical and Biophysical Research Communications*, **411**, 690-694. https://doi.org/10.1016/j.bbrc.2011.07.001

[61] Cao, J., *et al.* (2003) Expression of RANKL and OPG Correlates with Age-Related Bone Loss in male C57BL/6 Mice. *Journal of Bone and Mineral Research*, **18**, 270-277. https://doi.org/10.1359/jbmr.2003.18.2.270

[62] Titanji, K., *et al.* (2014) Dysregulated B Cell Expression of RANKL and OPG Correlates with Loss of Bone Mineral Density in HIV Infection. *PLOS Pathogens*, **10**, e1004497. https://doi.org/10.1371/journal.ppat.1004497

[63] Fischer, V. and Haffner-Luntzer, M. (2022) Interaction between Bone and Immune Cells: Implications for Postmenopausal Osteoporosis. *Seminars in Cell & Developmental Biology*, **123**, 14-21. https://doi.org/10.1016/j.semcdb.2021.05.014

[64] Gao, Y., *et al.* (2007) IFN-Gamma Stimulates Osteoclast Formation and Bone Loss *in Vivo* via Antigen-Driven T Cell Activation. *Journal of Clinical Investigation*, **117**, 122-132. https://doi.org/10.1172/JCI30074

[65] Cenci, S., *et al.* (2000) Estrogen Deficiency Induces Bone Loss by Enhancing T-Cell Production of TNF-Alpha. *Journal of Clinical Investigation*, **106**, 1229-1237. https://doi.org/10.1172/JCI11066

[66] Pacifici, R. (2012) Role of T Cells in Ovariectomy Induced Bone Loss—Revisited. *Journal of Bone and Mineral Research*, **27**, 231-239. https://doi.org/10.1002/jbmr.1500

[67] Buchwald, Z.S., *et al.* (2012) Osteoclast Activated FoxP3+ CD8+ T-Cells Suppress Bone Resorption *in Vitro*. *PLOS ONE*, **7**, e38199. https://doi.org/10.1371/journal.pone.0038199

[68] Izawa, T., *et al.* (2007) Crosstalk between RANKL and Fas Signaling in Dendritic Cells Controls Immune Tolerance. *Blood*, **110**, 242-250. https://doi.org/10.1182/blood-2006-11-059980

[69] Adkisson, H.D., *et al.* (2010) Immune Evasion by Neocartilage-Derived Chondrocytes: Implications for Biologic Repair of Joint Articular Cartilage. *Stem Cell Research*, **4**, 57-68. https://doi.org/10.1016/j.scr.2009.09.004

[70] Abe, S., Nochi, H. and Ito, H. (2017) Human Articular Chondrocytes Induce Interleukin-2 Nonresponsiveness to Allogeneic Lymphocytes. *Cartilage*, **8**, 300-306. https://doi.org/10.1177/1947603516661820

[71] Brudvik, K.W. and Tasken, K. (2012) Modulation of T Cell Immune Functions by the Prostaglandin E(2)-cAMP Pathway in Chronic Inflammatory States. *British Journal of Pharmacology*, **166**, 411-419. https://doi.org/10.1111/j.1476-5381.2011.01800.x

[72] MacKenzie, K.F., *et al.* (2013) PGE(2) Induces Macrophage IL-10 Production and a Regulatory-Like Phenotype via a Protein Kinase A-SIK-CRTC3 Pathway. *The Journal of Immunology*, **190**, 565-577. https://doi.org/10.4049/jimmunol.1202462

[73] Mahic, M., *et al.* (2006) FOXP3+CD4+CD25+ Adaptive Regulatory T Cells Express Cyclooxygenase-2 and Suppress Effector T Cells by a Prostaglandin E2-Dependent

Mechanism. *The Journal of Immunology*, **177**, 246-254. https://doi.org/10.4049/jimmunol.177.1.246

[74] Zhang, S., *et al.* (2018) Prostaglandin E(2) Hydrogel Improves Cutaneous Wound Healing via M2 Macrophages Polarization. *Theranostics*, **8**, 5348-5361. https://doi.org/10.7150/thno.27385

[75] Lohan, P., *et al.* (2016) Culture Expanded Primary Chondrocytes Have Potent Immunomodulatory Properties and Do Not Induce an Allogeneic Immune Response. *Osteoarthritis Cartilage*, **24**, 521-533. https://doi.org/10.1016/j.joca.2015.10.005

[76] Nakamura, H., *et al.* (2006) Enhanced Production of MMP-1, MMP-3, MMP-13, and RANTES by Interaction of Chondrocytes with Autologous T Cells. *Rheumatology International*, **26**, 984-990. https://doi.org/10.1007/s00296-006-0116-5

[77] Mohamad, S.F., *et al.* (2021) Neonatal Osteomacs and Bone Marrow Macrophages Differ in Phenotypic Marker Expression and Function. *Journal of Bone and Mineral Research*, **36**, 1580-1593. https://doi.org/10.1002/jbmr.4314

[78] Mohamad, S.F., *et al.* (2017) Osteomacs Interact with Megakaryocytes and Osteoblasts to Regulate Murine Hematopoietic Stem Cell Function. *Blood Advances*, **1**, 2520-2528. https://doi.org/10.1182/bloodadvances.2017011304

[79] Tucci, M., *et al.* (2011) Immature Dendritic Cells from Patients with Multiple Myeloma Are Prone to Osteoclast Differentiation *in Vitro*. *Experimental Hematology*, **39**, 773-783e1. https://doi.org/10.1016/j.exphem.2011.04.006

[80] Lee, J.H., *et al.* (2017) Tolerogenic Dendritic Cells Are Efficiently Generated Using Minocycline and Dexamethasone. *Scientific Reports*, **7**, Article No. 15087. https://doi.org/10.1038/s41598-017-15569-1