The Emerging Field of Osteoimmunology

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Abstract

Recent studies have elucidated unanticipated connections between the immune and skeletal systems, and this relationship has led to the development of a new field known as osteoimmunology. The goal of research in this field is to: 1) further understand how the bone microenvironment influences immune cell ontogeny and subsequent effector functions, and 2) translate basic science findings in bone biology to clinical applications for autoimmune diseases that target the skeleton such as rheumatoid arthritis. In this review, we will examine the recent findings of the interplay between the immune and skeletal systems. This discussion will focus on the cells and signaling pathways in osteoimmune interactions and how innate and adaptive immune effector cells as well as cytokines and chemokines play a role in the maintenance and dysregulation of skeletal-immune homeostasis. We will also discuss how immunomodulatory biologic drugs, which specifically target these cells and effector molecules, have transformed the treatment of autoimmune mediated inflammatory diseases (IMIDs) and metabolic bone diseases such as osteoporosis.

Keywords

Osteoimmunology; receptor-activator of nuclear factor kappa B (RANK); RANK-ligand; osteoprotegerin (OPG); arthritis; osteoporosis

Introduction

Bone appears to be a relatively static organ, there to provide structural support to the human form and serve as a niche for mesenchymal and hematopoietic progenitors. The skeleton, however, is anything but static as evidenced by the active process of bone remodeling which relies on a delicate balance between bone forming osteoblasts and bone resorbing osteoclasts (OCs) (1). The coordinated interplay of osteoblasts and OCs, known as coupling, continuously remodels bone through highly regulated molecular and cellular events such that the entire adult skeleton is replaced every ten years of human life (2). Disruption of the homeostatic balance of bone removal and replacement can manifest as pathologic bone loss observed in osteoporosis, periodontal disease, and some inflammatory arthritides, or as inappropriate new bone formation (1,3,4).

The dynamism of the skeleton is not limited to its perpetual turnover but can also be observed in the interactions between bone and other organ systems. One particularly intriguing interaction, which has gained much attention in recent years, is the interplay

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between the skeletal and immune systems. The recent understanding of a connection between innate and adaptive immune cells, immune system cytokines and chemokines, and cells involved in skeletal remodeling led to the development of a field known as osteoimmunology (5–8). This rapidly expanding field has the potential to facilitate the translation of basic science knowledge in bone biology into an improved pathophysiological understanding of many common, costly, and debilitating disorders.

In this review, we will discuss bone remodeling events as they relate to inflammatory and immune-mediated disorders. We will examine how these osteoimmune conditions can result in excess bone loss and, sometimes, pathologic new bone formation by reviewing major factors involved in bone turnover, both established and under current investigation. Lastly, the impact of immune system modulating therapy on pathologic bone resorption will be addressed.

Osteoblasts and Osteoclasts: Cellular Architects of Bone Formation and Remodeling

Osteoblasts are derived from pluripotent mesenchymal stem cells (MSC), which can also give rise to chondrocytes, myoblasts, tenocytes, neurons and adipocytes (6). During osteoblast differentiation, MSC express higher quantities of phenotypic markers like alkaline phosphatase and osteocalcin. MSC also express receptors for bone morphogenetic proteins (BMP) and the Wnt receptors low-density lipoprotein receptor related proteins (LRP) 5 and 6. It is activation of these receptors that promotes differentiation of the progenitors into bone-forming osteoblasts (4,9).

While osteoblasts are a pivotal half of the cellular remodeling component of the bone-remodeling unit, the first connection between the skeletal and immune systems we will describe concerns their bone resorbing counterparts, the OCs. OCs are giant, multinucleated cells uniquely designed to resorb bone. OCs precursors at or near the bone surface are induced to differentiate into OCs by factors in the surrounding environment, which we will discuss later. Binding of these factors induces the expression of several genes that are essential to OC function. The OC also undergoes structural changes to form a cell with a polarized shape. One side of the cell develops a ruffled border, which firmly attaches to the bone surface creating a sealing zone by rearrangement of the actin cytoskeleton. This creates an external vacuole into which hydrogen ions are secreted by the OC to acidify the microenvironment of the sealing zone. The OC also secretes tartrate-resistant acid phosphatase (TRAP) into the resorption space where it functions to decalcify bone. Staining for TRAP is a common technique used to identify OCs in culture or on sections of bone. The acidic environment created by secretion of hydrogen ions and TRAP helps to mobilize the mineral content of the bone. The OCs then secrete cathepsin K, which is involved in degradation of bone matrix exposed by the acid. The degradation products from this process are taken up by the OC and released on its apical surface into the circulation. Chief among these products are solubilized calcium and phosphate, which are essential to the homeostasis of the organism as a whole and are tightly balanced (1,3).

Osteoblast/Stromal Cells: the Nurse Mothers of the Hematopoietic System

While it has been well known that the vast majority of hematopoiesis occurs in the bone marrow, and that hematopoietic stem cell (HSC) proliferation and differentiation is controlled by “stromal” cells, the nature of these mesenchymal cells in the HSC niche has been poorly understood. Recent work in this area has revealed that the stromal cells responsible for HSC regulation in bone marrow are bona fide osteoblasts (10). Interestingly, this discovery was made by bone biologists who were studying the effects of parathyroid

hormone (PTH), whose primary endocrine function is to maintain blood calcium homeostasis through osteoblast-osteoclast coupling and renal absorption. By analyzing the phenotype of transgenic mice that express a constitutive PTH receptor in bone forming osteoblasts (Col1-caPTHR) (11), the investigators demonstrated that osteoblastic cells produce high levels of the Notch ligand jagged 1 and support an increase in HSC. Furthermore, PTH increased: i) osteoblast numbers in stromal cultures, ii) augmented ex vivo HSC growth, iii) HSC numbers in vivo, and iv) survival of wild-type mice after lethal irradiation and bone marrow transplantation. Thus, osteoblastic cells are a regulatory component of the hematopoietic stem cell niche in vivo that influences stem cell function through Notch activation. Moreover, since recombinant PTH (teraparitide) is an approved anabolic therapy for osteoporosis (12), researchers are now investigating its potential as a HSC therapy to treat anemia and general immunosuppression.

Monocytes, Macrophages, and Dendritic Cells: Innate Immune Sources of Osteoclast Precursors

Unlike their MSC-derived osteoblast counterparts, OCs are derived from hematopoietic cells. These hematopoietic-lineage cells also generate immune cells such as lymphocytes, phagocytes, and dendritic cells (DCs). OCs specifically derive from the myeloid-monocyte branch of hematopoietic cells, and thus, they share the same precursor as macrophages and myeloid DCs (13). Thus, it is the microenvironment to which the common myeloid precursor cell is exposed that will determine whether they differentiate into innate immune cells or bone resorbing cells. The development of OCs from their precursor cells has been studied by flow cytometric immunophenotyping of surface proteins. The multipotential myeloid progenitor cell population is defined as positive for the surface marker c-Kit. This population moderately expresses a pan-myeloid lineage marker CD11b, and is negative for c-Fms, which is the tyrosine kinase receptor for macrophage colony stimulating factor (M-CSF) — needed to prime cells for OC differentiation.

Our group has previously shown that the frequency of cells expressing the pan-myeloid lineage marker CD11b is elevated in mice and humans with inflammatory arthritis (14,15). These CD11b+ cells mature into two major subpopulations of monocytes within the bone marrow (16,17). There is a classical monocyte subpopulation and a non-classical monocyte subpopulation. The classical monocyte subpopulation is characterized in humans as $CD14^{hi}CD16^{-}$. In mice, they are $CD11b^{+}Gr1^{hi}$. These cells make up about 90–95% of monocytes in humans. The non-classical monocyte subpopulation makes up about 5–10% of monocytes and is characterized as $CD14^{+}CD16^{+}$ in humans and $CD11b^{+}Gr1^{lo}$ in mice (16). These non-classical monocytes show a considerable increase in number during a variety of systemic infectious conditions. A link between these innate immune cells and bone disease is evidenced by the increased percentage of non-classical, pro-inflammatory monocytes found in the blood of rheumatoid arthritis (RA) patients, particularly during active disease (18). This increase correlated with erythrocyte sedimentation rates and C-reactive protein levels (19). Transforming growth factor-beta (TGF-β), a cytokine found in rheumatoid synovial fluid, induced CD16 expression on CD14+ monocytes. Therapy for RA resulted in a decrease in the $CD14^{+}CD16^{+}$ monocyte subpopulation (20).

Upon interaction of the CD11b+ monocytic precursor with stem cell factor (SCF), they become positive for the M-CSF receptor c-Fms (21). C-Fms is a key determinant of development for cells in the monocyte-macrophage lineage (1). Thus, the multipotential progenitor cell is designated $c\text{-}Kit^{+}$ $CD11b^{dull}$ $c\text{-}Fms^{-}$ while the early-stage precursor is $c\text{-}Kit^{+}$ $CD11b^{dull}c\text{-}Fms^{+}$. The presence of M-CSF converts the early-stage precursor cells to late-stage precursors by triggering increased CD11b expression and also by leading to upregulated surface expression of receptor-activator of NFκB (RANK) to which RANK

ligand (RANKL) will bind in order to begin the cascade of signaling events which culminate in OC formation (21).

RANK signaling in OC precursors (OCP) occurs through TRAF6 (TNF receptor associated factor 6) (22–27). The importance of TRAF6 is highlighted by the fact that TRAF6 knockout mice are osteopetrotic, TRAF6 has various downstream mediators that regulate the expression of genes specific for OC differentiation and activation including NFκB, AP-1 mediated by JNK pathway, cascades of MAPK such as TGF-β-inducible kinase TAK1 and the p38 stress kinase (28). The result of NFκB and c-Fos activation is the induction of NFATc1, a transcription factor, which leads ultimately to the increased expression of the genes for TRAP, cathepsin K, DC-STAMP and other genes essential for OC formation and function.

Though our discussion of osteoimmune interactions began with the OC and its origins, the osteoblast plays a key role in potentiating the osteoimmune interactions of OCs by virtue of the fact that RANKL is expressed by osteoblasts in the bone marrow stromal environment in response to hormones like vitamin D3, parathyroid hormone, and estrogen (3,8). Interestingly, RANKL was discovered by immunologists who were looking for novel ligands from T cells that were induced by DCs in mixed leukocyte reactions, as tumor necrosis factor (TNF)-related activation-induced cytokine (TRANCE) (29). Anderson et al also discovered the gene for this cytokine in a screen to identify the ligand for RANK, which they cloned as a co-stimulatory receptor on DC (30). Thus, TRANCE or RANKL, was considered to be a dendritic cell-restricted survival factor important in T cell-dendritic cell interactions. Around the same time, researchers were trying to identify an OC differentiating factor (ODF). Based on their prior work that identified osteoprotegerin (OPG) as an osteoblast-derived secreted member of the TNF receptor family that inhibited osteoclastogenesis as a receptor antagonist, investigators at Amgen Inc. discovered OPG-ligand (OPGL) (31,32). Yasuda et al. also identified this long sought after ODF via expression cloning for a ligand that bound to OPG (33). Thus, RANKL as it is known today, was independently identified as ODF, TRANCE, and OPGL, by immunologists and bone biologists, and these discoveries are recognized as the keystone of osteoimmunology.

In addition to monocytes, macrophages are another innate immune system cell type that can also serve as a source of OC (34). Macrophages are mononuclear cells of the myeloid lineage, which are present in a number of tissues normally maintaining homeostasis and aiding in repair. One interesting property of macrophages is their ability to fuse with each other to form multinucleated cells. The multinucleated cells, or polykaryons, formed from macrophages allow the cell to resorb large materials that the mononuclear pre-fusion cells would not be able to. This ability has put macrophages at a central point in the evolution and function of the immune and skeletal systems because multinucleated macrophages form giant cells in chronic inflammatory sites like granulomas, and they also form OCs in the bone. How a macrophage determines whether to become a giant cell or an OC upon fusion is governed by the local cytokine milieu with RANKL pushing bone-marrow derived macrophages to form OCs while interleukin-4 drives giant cell formation (35).

A molecule that has recently gained importance in the fusion process and also serves to be another pivotal molecule in the field of osteoimmunology is the dendritic cell-specific transmembrane protein (DC-STAMP). DC-STAMP was initially identified in myeloid DCs as a 53 kDa, interleukin-4 (IL-4)-inducible, seven-member transmembrane protein with no considerable sequence homology to other proteins (36,37). DC-STAMP has also been found in macrophages and OCs (38–40). To-date, its ligand and its function in myeloid DCs is unknown, although recent studies with mice genetically deficient for DC-STAMP suggest a potential role in autoimmunity since DC-STAMP$^{-/-}$ mice exhibit DCs with higher

phagocytic and antigen presentation activity via upregulated MHC class I and II pathways (41). In bone marrow-derived macrophages, DC-STAMP is essential for multinucleation to form giant cells in the presence of IL-4, and OCs in the presence of RANKL and M-CSF (38–40). In support of the importance of DC-STAMP in osteoclastogenesis are the findings that DC-STAMP$^{-/-}$ mice are osteopetrotic, and they do not have OCs (42,43). That DC-STAMP plays a potential role in autoimmunity and is essential for osteoclastogenesis suggests that further research may uncover its position as another link between the skeletal and immune systems.

Myeloid DCs represent another potential innate immune cell that shares a common precursor with the OC and macrophage. They form after exposure of monocytic precursor to GM-CSF and IL-4 (13). DCs function in large part as antigen-presenting cells, but have other functions and are distributed throughout the body. Since DCs express RANK and can be found in the joints of rheumatoid arthritis patients, it is thought that DCs mediate osteoimmune interactions indirectly by activating T cells to produce RANKL which can then lead to osteoclastogenesis (29,44). Though GM-CSF and IL-4 have been shown to inhibit OC formation by effects on c-fos and STAT-6 respectively, there is some recent evidence that uncommitted cells that have started down the path of forming DCs can transdifferentiate to become OCs (13,45). One group has found TRAP expression among monocyte-derived immature DCs. When these cells were treated with M-CSF and RANKL, they became multinucleated, large cells. These cells did not express the human DC marker CD1a nor did they express the monocyte marker CD14. Despite the absence of CD14 on the fully transdifferentiated dendritic-cell derived OCs, an intermediate CD14+ proliferating stage was identified among these cells. This cell type expressed both DC markers (like CD1a, CD80, CD86, CD11c, HLA-DR) and monocyte/macrophage cell surface markers (like CD11b, CD14, CD16). The DC and monocyte/macrophage markers were found to be down-regulated with cell fusion. The authors of the study identifying the dendritic-cell derived OCs hypothesized that the transdifferentiation process occurs in two steps. Firstly, the cells proliferate and give rise to an intermediate, which can express both macrophage and DC markers. Secondly, the macrophage and DC markers are down-regulated along with cessation of cell division and onset of fusion. The authors further state that the immature DCs must use transcription factors other than c-fos when generating OCs via transdifferentiation and that this process only becomes apparent in chronic inflammatory states when differentiation into DCs is occurring at a high enough rate for the low-level of steady state transdifferentiation to become magnified. They posit that during rheumatoid arthritis, for example, the autoimmune inflammatory response is driven by perivascular DCs which present self-antigen to autoreactive T cells. These T cells then release joint-damaging factors. The DCs additionally would induce RANKL expression on T cells and induce osteoclastogenesis. A second population of DCs would be immature in nature and have the capacity to transdifferentiate into bone-resorbing OCs in the presence of the M-CSF and RANKL in the rheumatoid synovial fluid (46).

Cytokines as Mediators of Crosstalk between the Skeleton and Immune Cells

In addition to M-CSF and RANKL, other cytokines upregulated during inflammation and immune responses play a role in determining the fate of precursor cells capable of generating OCs. One of this most important is TNF, which is an inflammatory cytokine produced by macrophages, neutrophils, keratinocytes, endothelial cells and fibroblasts with a myriad of effects from promoting cell-proliferation to facilitating apoptosis. Production of TNF is driven by NFκB response elements and its expression is largely regulated by post-transcriptional modifications of AU-rich elements (ARE) in the 3′ region of the TNF gene. Resting cells have lower expression of TNF because the ARE mediate the degradation of

TNF mRNA. After cellular activation, the TNF mRNA is stabilized and expression levels can increase 200-fold. Over expression of TNF following cellular activation is prevented by the activity of tristetraprolin, which degrades the activation-induced TNF mRNA. Deregulation of the regulation of TNF expression following cellular activation can lead to chronically elevated TNF levels (47).

The link between deregulated TNF and inflammatory arthritis came out of observations that this cytokine is elevated in the synovial fluid and synovial membrane of RA and PsA patients (15). In this context, TNF can cause joint inflammation and trigger cartilage destruction. Important to its role in altering bone remodeling is the pro-osteoclastogenic effect of TNF (48). TNF can stimulate osteoclastogenesis via its interaction with the p55 subunit of the TNF receptor (TNFp55r) (48). Upon binding to this receptor, TNF exerts several effects that foster increased OC formation. TNF stimulates RANKL expression in bone marrow stromal cells and also activates the p38 MAPK cell-signaling pathway, which leads to increased M-CSF receptor c-Fms expression.

In-vivo animal studies have also captured the importance of TNF in the development of autoimmune and inflammatory erosive arthritis. The TNF-transgenic mouse, for example, closely mimics human disease and represents the first predictive animal model of arthritis as these animals develop erosive arthritis with focal subchondral and joint margin bone erosions (49). On a cellular level, an effect of TNF in these animals is a four to seven-fold increase in the frequency of $CD11b^{hi}$ cells in peripheral tissues like spleen and blood that can serve as OCP. The increase in this cell population coincided with the time at which TNF levels increased in these transgenic animals. Furthermore, treatment of the TNF transgenic mice with anti-TNF agents restored the number of cells in this population to levels seen in their wild type littermates (14).

Recent studies show that TNF increases expression of the glycoprotein dickkopf-1 (DKK-1), a natural antagonist of the Wnt pathway in osteoblastogenesis. $DKK\text{-}1^{+/-}$ mice have high bone mass and increased expression in transgenic mice leads to osteopenia (9). It was recently shown that TNF-induced DKK-1 expression in inflammatory arthritis has two major consequences (50). Increased DKK-1 expression impairs bone-forming osteoblast development and elevated expression also suppresses the production of OPG. Taken together, DKK-1 favors osteoclastic bone resorption both by suppression of OPG and by inhibition of bone formation, and synergizes with TNF to mediated bone loss. In terms of osteophyte formation, TNF blockade in the face of increasing concentrations of DKK-1 blockade did not show any difference relative to DKK-1 blockade alone (50). Thus, TNF is a potent inhibitor of bone formation as well as a driver of bone resorption.

Th17: a Central Effector Cell of Osteoimmunology

The importance of bone to lymphocytes has long been recognized, as the early development of lymphocytes is known to take place in the bone marrow. However, the idea that lymphocytes could be involved in bone remodeling was unheard of until the aforementioned discovery of TRANCE, OPGL, ODF, and RANKL, and that the predominant phenotype of transgenic mice defective in RANK-RANKL signaling is severe osteopetrosis due to the complete absence of OCs (51). Using these knockout mice in models of inflammatory bone loss, it has been demonstrated that T-cell derived RANKL is responsible for pathologic osteoclastogenesis and focal erosion (52). However, there are always some activated T cells in the immune system but no bone loss under normal T cell response. This is because T cells also have a negative regulatory mechanism for inhibiting osteoclastogenesis. Activated T cells also secret interferon γ and IL-4 that inhibit RANK. Interferon γ binds to the interferon γ receptor on OC, activates the ubiquitin proteosome pathway to degrade the

adapter protein TRAF6 (8). IL-4 reduces NFκB nuclear translocation by inhibiting IkκB phosphorylation, thus markedly inhibiting NFκB DNA binding activity and blocking osteoclastogenesis entirely (53). Moreover, when DCs present antigen to naive T cells, the activated T cells express RANKL, which provides further signaling to DCs. The DCs negatively regulate RANKL-RANK signaling by up regulating the production of RANKL decoy receptor OPG that competitively inhibits RANKL binding to RANK (6).

Based on the positive and negative regulation of T cells on OC function, we might expect that T cells are important in the balance of bone metabolism under non-pathological conditions and contribute to bone destruction in pathological conditions by a different mechanism. It is reported that activated T cells can directly trigger osteoclastogenesis through RANKL, and systemic activation of T cells in vivo leads to a RANKL-mediated increase in osteoclastogenesis and bone loss (52). However, T cells are not absolutely needed for osteoclastogenesis in some models of inflammatory bone destruction. It is reported in the collagen-induced arthritis RA model that mice lacking T and B lymphocytes can still develop arthritic lesions after immunization with type II collagen; therefore, T cells might play a role in initiation of inflammation or exaggerating bone loss (54).

Th17 cells, a new CD4+ T helper cell subset, were found to play critical role in the pathogenesis of RA. Th17 cells, so-named for their production of IL-17, are generated via a lineage distinct from the Th1 and Th2 lineages (55). It has been shown that TGF-β, IL-6, IL-21 and IL-23 are involved in promoting differentiation of Th17 cells from CD4+ T cells in peripheral blood (56,57). The effector cytokines associated with Th17 include IL-17, IL-21 and IL-22 (58). Th17 cells have also been found to be crucial mediators for cross talk between immune system and other tissues, and are involved in inflammation, organ-specific autoimmunity and tissue damage.

IL-17, produced by Th17 cells, is involved in promoting the expression of many proinflammatory cytokines, chemokines and other mediators that contribute to inflammation and the erosion of cartilage and bone in RA. It triggers the activation of NF-κB and p38 MAP kinase on epithelial, endothelial, and fibroblastic stromal cells. This activation results in the secretion of IL-1, TNF, IL-6, IL-8, and prostaglandin E2 (59). IL-17 demonstrates synergistic effects with IL-1 and TNF in inducing joint inflammation and cartilage destruction (60). It is a potent inducer of RANK, and it also has the capacity to induce joint destruction in an IL-1-independent manner (60). It is reported that blockade of IL-17 with an IL-17 receptor/human IgG1-Fc fusion protein prior to disease onset attenuates disease and reduces joint damage in the rat adjuvant-induced arthritis (AIA) (61). Also, treatment with a neutralizing anti-murine IL-17 antibody after the onset of collagen-induced arthritis reduces joint inflammation, cartilage destruction, and bone erosion (62). Thus, based on these studies, Th17 cell might be a central effector in the erosion of cartilage and bone in joint disease. From these results, it is possible that IL-17 therapy may be a promising therapeutic target for rheumatoid arthritis.

By comparing functional IL-17 levels in synovial explant cultures from RA, OA and normal joints, it was found that IL-17 is spontaneously produced in RA synovium (63). It was also found that IL-17 in synovial fluid from RA patients was significantly higher than that in OA patients. IL-17 in synovial fluid is a potent stimulator of osteoclastogenesis while anti-IL-17 antibody administration significantly inhibited OC formation in RA synovial tissues (64). In one study, Th17 cell number as well as IL-17 levels in peripheral blood (PB), synovial fluid (SF) and synovial tissue (ST) were quantified in sample from patients with RA, OA and in healthy controls (65). The percentage of Th17 cells in RASF was found to be significantly higher than that in normal SF and RAPB with or without stimulation by PMA/I plus IL-23. After stimulation with PMA/I plus IL-23, Th17 cell number was significantly increased in

both normal and RAPB and RASF. RT-PCR shows that *il-17* gene expression is comparable in healthy control and OA tissue, while it is significantly increased in RAST. Since Th17 cells respond to IL-23, the study also examined the *il-23* level in RASF after observing the high number of Th17 cells in RASF. *Il-23* was rarely detected in RASF, while it was 4.6 times higher in macrophages from RA joints compared to controls. Since IL-27 and IFN-γ suppress development of Th17 cells, their mRNA levels were also examined in RASF macrophages. Like *il-23*, *il-27* and *ifn*-γ mRNA levels were found to be significantly higher in RA-joint macrophages.

B Cells in osteoimmunology

Unlike T cells that directly influence osteoclastogenesis by expressing RANKL, B cells influence skeletal health primarily through indirect mechanisms. One important role of B cells in RA is that they are the source of autoantibodies such as rheumatoid factor (RF). RF is an antibody against the Fc portion of IgG, which is itself an antibody. RF and IgG join to form immune complexes, which contribute to the disease process. Activated B cells with RF specificity are abundant in rheumatoid synovial membrane, and RF is detected in about 75% of patients with RA (66). Although RF is considered as a serological marker of RA, about 20% of RA patients do not have RF in their blood, and the level of RF does not correlate perfectly with the severity of disease (67).

B cells may function as antigen presenting cells to present antigen to CD4+ T cells, and they provide signals for T cell clonal expansion and effector function. About 30% of RA patients develop synovitis with infiltrated B cells and T cells that aggregate into lymphocyte follicles with germinal center formation (68). To find out the activation requirement of the follicular CD4+ T cells, Takamura et al. isolated the CD4+ T cells residing in synovial tissue T cell/B cell follicles by microdissection and analyzed them by adoptive transfer (69). They found that CD4+ T cells from independent, nonadjacent follicles have identical T cell receptor (TCR) sequences, indicating recognition of the same antigen in different germinal centers. The criteria for functional adoptively transferred CD4+ T cells are matching of major histocompatability complex (MHC) class II polymorphisms and presence of B cells. Synovial tissue infiltrated by T cells, macrophages, and DCs, but not by B cells failed to support the activation of adoptively transferred CD4+ T cell clones, suggesting that B cells are critical in T cell activation by providing the relevant antigen. The anti-CD20 B cell depletion study in this paper also shows that B cells could not substitute in maintaining T cell activation.

B cells in RA synovial membrane may also function by secreting pro-inflammatory cytokines. Activated B cells, through signals from the B cell receptor (BCR) and CD40, produce high levels of IL-6, lymphotoxin and TNF that amplify T cell reactions and promote germinal center formation in inflamed tissue(70). In contrast, B cells activated by CD40 stimulation alone, without specific antigen recognition, produce high levels of IL-10 that serves to suppress local immune responses instead of pro-inflammatory cytokines (70). IL-6 and IL-10 produced by B cells stimulate further activation of B cells through a positive feedback mechanism.

Bone marrow infiltration is composed mostly by lymphocytes with more than 80% of them being B cells. In one RA model, B cells were found to infiltrate bone marrow near arthritis lesions (71). In the hTNF-Tg mouse, which is a chronic RA model, lymphocyte infiltration was found in the bone marrow of juxta-articular bone lesions, which are caused by invasion of hyperplasitic synovium into the joint space. Bone marrow B cells in these infiltrates have been found to be actively involved in the healing of bone lesions. Bone marrow B cells generate BMP to induce endosteal bone formation.

Another discovery of the role of B cell in osteoimmunology is that B cells cooperate with T cells to play a role in basal bone turnover (72). It is reported that B cells play critical role in regulating bone homeostasis by their OPG production, and the OPG production by B cells is promoted by T cell via CD40-CD40L costimulation. In this study, bone marrow B cells subpopulations were isolated by immuno-magnetic isolation and quantification of OPG production level of those cells by ELISA and RT-PCR showed that B cells are the major source of OPG in bone marrow, accounting for 64% of total OPG production in bone marrow with 45% derived from mature B cells (72). B cell knockout mice exhibit significant reductions in OPG levels and elevated bone resorption. This phenotype can be rescued by B cell reconstitution. OPG from splenic B cells, although a smaller proportion than that in bone marrow and peripheral blood, can be up-regulated by CD40L stimulation. Moreover, reduced B cell OPG secretion and increased bone loss are observed in T cell-deficient, nude, CD40 knockout, and CD40L knockout mice, which suggests that B cell-secreted OPG is augmented by B and T cell crosstalk via CD40-CD40L. Their results show that OPG produced by B cells may serve to maintain the balance of OPG and RANKL, thereby maintaining the rate of bone turnover (72).

Biologic Therapies that Target Osteoimmunology

TNF inhibitors have been used with remarkable success in the treatment of osteoimmune arthritis. Extensive literature exists on their successful use in RA; however, other inflammatory arthritides have also been well controlled by TNF blockade. In a randomized placebo controlled clinical trial, nearly 90% of psoriatic arthritis (PsA) patients showed clinical improvement according to the PsA Response Criteria (PsARC) when given etanercept, a recombinant soluble TNF receptor. Other studies on etanercept and infliximab, a chimeric monoclonal anti-TNF antibody, showed similar degrees of improvement in joint inflammation in PsA patients relative to those receiving placebo. In a placebo-controlled phase-3 study using 25 mg etanercept administered subcutaneously twice weekly, joint space narrowing and erosions were halted in the treatment group compared to the control group (73). A phase-3 study of infliximab showed inhibition of radiographic disease progression at 6 months of treatment (74). A third anti-TNF agent approved for treatment of PsA is adalimumab, the fully human anti-TNF monoclonal antibody given subcutaneously at 40 mg every other week or weekly. In a phase 3 study of this agent, radiographic progression of disease as identified by hand and foot x-rays was significantly inhibited (75).

Rituximab is a chimeric anti-CD20 monoclonal antibody that depletes pre- and mature B cells by complement-dependent cytotoxicity and antibody-dependent cell-mediated cytotoxicity. Rituximab is an approved treatment for B cell lymphoma. The rationale of using rituximab for RA is that in RA patients there are autoantibody producing B cells that mediate disease. By eliminating B cells and letting the immune system regenerate B cells, the possibility of the autoantibody producing B cells to survive is very small (67). In 2002, Edwards et al. presented data on efficacy and safety results of rituximab in 122 randomized RA patients treated with rituximab combined with methotrexate (MTX a conventional anti-inflammatory agent) or MTX alone (76). The results showed that more than 40% of those receiving rituximab with MTX had improvements in symptoms at the end of 24 weeks, compared to just 13% of those receiving MTX alone. Though the rationale of rituximab is to deplete autoantibody producing B cells, the clinical efficacy seen in these patients does not directly correlate with decreases in serum immunoglobulin levels (IgG, IgM, and IgA) including autoantibodies and rheumatoid factor (77). Thus, B cells must have other pathological functions in RA besides producing autoantibodies; this remains an active area of investigation.

T cell activation requires both antigen recognition by the T cell receptor and costimulation by interactions of CD28 on the T cell and CD80 or CD86 on the antigen presenting cell (6). After activation of the T cell, cytotoxic T-lymphocyte antigen 4 (CTLA-4) is induced and negatively regulates T cell activation by binding to CD80 or CD86 with a higher avidity than CD28. Thus, CTLA-4 became an attractive molecule to study in designing a T cell-blocking agent. A soluble CTLA-4 immunoglobulin, known as abatacept, was developed to block costimulation of T cells thereby halting normal T cell activation. Several studies have examined the use of abatacept in the treatment of RA. A one-year, multicenter, double-blinded, placebo-controlled trial of 339 subject showed that patients receiving 10 mg/kg of abatacept had 20% improvement was obtained according to American College of Rheumatology criteria (ACR 20) compared to patients receiving placebo. Abatacept in combination with other disease modifying anti-rheumatic drugs (DMARDs) was compared to placebo plus DMARDs in RA patients who did not respond to anti-TNF therapy in a 6-month, double-blinded, randomized, placebo-controlled trial. At the end of the trial, abatacept treatment led to a higher proportion of patients achieving the ACR 20, 50, and 70 improvement criteria compared to those receiving placebo. Another study showed significant reduction in the progression of radiographically observable joint erosions and joint space narrowing in patients receiving abatacept compared to placebo at one year of follow up (78). Recent data shows that CTLA-4 can act directly on OC precursors by inhibiting RANKL- and TNF-mediated osteoclastogenesis in a dose-dependent manner in the absence of T cells in vitro. Furthermore, this inhibitory effect was seen in a T cell independent TNF animal model of arthritis where osteoclastogenesis and inflammatory bone erosion development were halted by CTLA-4. Thus, CTLA-4-Ig, abatacept, has both indirect and direct effects on OC development by its actions on T cell activation and OC precursor differentiation (79).

In vivo data shows that RANKL inhibition leads to OC apoptosis and that no in vivo bone models are refractory to RANKL inhibition. Thus, anti-RANKL therapy has been pursued as a treatment for osteoimmunologic conditions (80). Since OPG is considered a natural decoy receptor for RANKL, recombinant Fc-OPG therapy was initially tried as a means of inhibiting RANKL. This therapy was initially successful, but it was thought that it may affect tumor surveillance by its binding to TRAIL (81). A safer alternative was the generation of an anti-RANKL humanized monoclonal antibody known as denosumab (82). Denosumab is non-cytotoxic with a high affinity for human RANKL. Several clinical trials have been carried out on denosumab. In trials for osteoporosis, metastatic breast cancer, and multiple myeloma (a disorder where monoclonal plasma cells proliferate and lytic bone lesions are a common clinical sign), denosumab was shown to have rapid onset that could be sustained up to half a year in some patients. A trial of denosumab in RA showed that patients receiving a dose of 180 mg had significantly fewer bone erosions at half a year compared to placebo as assessed by MRI. Another study found increased bone mineral density in several areas as well as decreased markers of bone resorption in RA patients receiving denosumab (80).

Conclusions

Given that clinicians have been treating steroid-induced osteoporosis in patients taking medicine for autoimmune disease for over 60 years, it is somewhat surprising that significant collaborative research between immunologists and bone biologist has only recently begun. While there may be many reasons for this, two major obstacles have been the lack of overlapping molecular genetic pathways and research question that require the investigators to evaluate both systems to derive meaningful information. Now that these obstacles have been removed, and biologic therapies that target the immune and musculoskeletal systems simultaneously have been developed, there is great potential for

advances in osteoimmunology that will transform our understanding of human health and disease, and provide the new medicines for the 21st century.

References

1. Teitelbaum SL. Osteoclasts: What do they do and how do they do it? Am J Pathol. 2007; 170:427–435. [PubMed: 17255310]
2. Rodan GA, Martin TJ. Therapeutic approaches to bone diseases. Science. 2000; 289:1508–1514. [PubMed: 10968781]
3. Boyle WJ, Simonet WS, Lacey DL. Osteoclast differentiation and activation. Nature. 2003; 423:337–342. [PubMed: 12748652]
4. Lories RJ, Derese I, Luyten FP. Modulation of bone morphogenetic protein signaling inhibits the onset and progression of ankylosing enthesitis. J Clin Invest. 2005; 115:1571–1579. [PubMed: 15902307]
5. Lorenzo J, Choi Y. Osteoimmunology. Immunol Rev. 2005; 208:5–6. [PubMed: 16313336]
6. Walsh MC, Kim N, Kadono Y, Rho J, Lee SY, Lorenzo J, Choi Y. Osteoimmunology: Interplay between the immune system and bone metabolism. Annu Rev Immunol. 2006; 24:33–63. [PubMed: 16551243]
7. Takayanagi H. Osteoimmunology: Shared mechanisms and crosstalk between the immune and bone systems. Nat Rev Immunol. 7:292–304. [PubMed: 17380158]
8. Rho J, Takami M, Choi Y. Osteoimmunology: Interactions of the immune and skeletal systems. Mol Cells. 2004; 17:1–9. [PubMed: 15055519]
9. Yamaguchi A, Komori T, Suda T. Regulation of osteoblast differentiation mediated by bone morphogenetic proteins, hedgehogs, and Cbfa1. Endocr Rev. 2000; 21:393–411. [PubMed: 10950158]
10. Calvi LM, Adams GB, Weibrecht KW, Weber JM, Olson DP, Knight MC, Martin RP, Schipani E, Divieti P, Bringhurst FR, Milner LA, Kronenberg HM, Scadden DT. Osteoblastic cells regulate the haematopoietic stem cell niche. Nature. 2003; 425:841–846. [PubMed: 14574413]
11. Calvi LM, Sims NA, Hunzelman JL, Knight MC, Giovannetti A, Saxton JM, Kronenberg HM, Baron R, Schipani E. Activated parathyroid hormone/parathyroid hormone-related protein receptor in osteoblastic cells differentially affects cortical and trabecular bone. J Clin Invest. 2001; 107:277–286. [PubMed: 11160151]
12. Neer RM, Arnaud CD, Zanchetta JR, Prince R, Gaich GA, Reginster JY, Hodsman AB, Eriksen EF, Ish-Shalom S, Genant HK, Wang O, Mitlak BH. Effect of parathyroid hormone (1–34) on fractures and bone mineral density in postmenopausal women with osteoporosis. N Engl J Med. 2001; 344:1434–1441. [PubMed: 11346808]
13. Miyamoto T, Ohneda O, Arai F, Iwamoto K, Okada S, Takagi K, Anderson DM, Suda T. Bifurcation of osteoclasts and dendritic cells from common progenitors. Blood. 2001; 98:2544–2554. [PubMed: 11588053]
14. Li P, Schwarz EM, O'Keefe RJ, Ma L, Looney RJ, Ritchlin CT, Boyce BF, Xing L. Systemic tumor necrosis factor alpha mediates an increase in peripheral CD11bhigh osteoclast precursors in tumor necrosis factor alpha-transgenic mice. Arthritis Rheum. 2004; 50:265–276. [PubMed: 14730625]
15. Ritchlin CT, Haas-Smith SA, Li P, Hicks DG, Schwarz EM. Mechanisms of TNF-alpha- and RANKL-mediated osteoclastogenesis and bone resorption in psoriatic arthritis. J Clin Invest. 2003; 111:821–831. [PubMed: 12639988]
16. Strauss-Ayali D, Conrad SM, Mosser DM. Monocyte subpopulations and their differentiation patterns during infection. J Leukoc Biol. 2007
17. Gordon S, Taylor PR. Monocyte and macrophage heterogeneity. Nat Rev Immunol. 2005; 5:953–964. [PubMed: 16322748]
18. Kawanaka N, Yamamura M, Aita T, Morita Y, Okamoto A, Kawashima M, Iwahashi M, Ueno A, Ohmoto Y, Makino H. CD14+, CD16+ blood monocytes and joint inflammation in rheumatoid arthritis. Arthritis Rheum. 2002; 46:2578–2586. [PubMed: 12384915]

19. Wijngaarden S, van Roon JA, Bijlsma JW, van de Winkel JG, Lafeber FP. Fcgamma receptor expression levels on monocytes are elevated in rheumatoid arthritis patients with high erythrocyte sedimentation rate who do not use anti-rheumatic drugs. Rheumatology (Oxford). 2003; 42:681–688. [PubMed: 12709546]
20. Ziegler-Heitbrock L. The CD14+ CD16+ blood monocytes: Their role in infection and inflammation. J Leukoc Biol. 2007; 81:584–592. [PubMed: 17135573]
21. Arai F, Miyamoto T, Ohneda O, Inada T, Sudo T, Brasel K, Miyata T, Anderson DM, Suda T. Commitment and differentiation of osteoclast precursor cells by the sequential expression of c-fms and receptor activator of nuclear factor {kappa}B (RANK) receptors. J Exp Med. 1999; 190:1741–1754. [PubMed: 10601350]
22. Kim HH, Lee DE, Shin JN, Lee YS, Jeon YM, Chung CH, Ni J, Kwon BS, Lee ZH. Receptor activator of NF-kappaB recruits multiple TRAF family adaptors and activates c-jun N-terminal kinase. FEBS Lett. 1999; 443:297–302. [PubMed: 10025951]
23. Armstrong AP, Tometsko ME, Glaccum M, Sutherland CL, Cosman D, Dougall WC. A RANK/TRAF6-dependent signal transduction pathway is essential for osteoclast cytoskeletal organization and resorptive function. J Biol Chem. 2002; 277:44347–44356. [PubMed: 12185073]
24. Naito A, Yoshida H, Nishioka E, Satoh M, Azuma S, Yamamoto T, Nishikawa S, Inoue J. TRAF6-deficient mice display hypohidrotic ectodermal dysplasia. Proc Natl Acad Sci U S A. 2002; 99:8766–8771. [PubMed: 12060722]
25. Lomaga MA, Yeh WC, Sarosi I, Duncan GS, Furlonger C, Ho A, Morony S, Capparelli C, Van G, Kaufman S, van der Heiden A, Itie A, Wakeham A, Khoo W, Sasaki T, Cao Z, Penninger JM, Paige CJ, Lacey DL, Dunstan CR, Boyle WJ, Goeddel DV, Mak TW. TRAF6 deficiency results in osteopetrosis and defective interleukin-1, CD40, and LPS signaling. Genes Dev. 1999; 13:1015–1024. [PubMed: 10215628]
26. Kobayashi N, Kadono Y, Naito A, Matsumoto K, Yamamoto T, Tanaka S, Inoue J. Segregation of TRAF6-mediated signaling pathways clarifies its role in osteoclastogenesis. EMBO J. 2001; 20:1271–1280. [PubMed: 11250893]
27. Ye H, Arron JR, Lamothe B, Cirilli M, Kobayashi T, Shevde NK, Segal D, Dzivenu OK, Vologodskaia M, Yim M, Du K, Singh S, Pike JW, Darnay BG, Choi Y, Wu H. Distinct molecular mechanism for initiating TRAF6 signalling. Nature. 2002; 418:443–447. [PubMed: 12140561]
28. Wong BR, Besser D, Kim N, Arron JR, Vologodskaia M, Hanafusa H, Choi Y. TRANCE, a TNF family member, activates Akt/PKB through a signaling complex involving TRAF6 and c-src. Mol Cell. 1999; 4:1041–1049. [PubMed: 10635328]
29. Wong BR, Josien R, Lee SY, Sauter B, Li HL, Steinman RM, Choi Y. TRANCE (tumor necrosis factor [TNF]-related activation-induced cytokine), a new TNF family member predominantly expressed in T cells, is a dendritic cell-specific survival factor. J Exp Med. 1997; 186:2075–2080. [PubMed: 9396779]
30. Anderson DM, Maraskovsky E, Billingsley WL, Dougall WC, Tometsko ME, Roux ER, Teepe MC, DuBose RF, Cosman D, Galibert L. A homologue of the TNF receptor and its ligand enhance T-cell growth and dendritic-cell function. Nature. 1997; 390:175–179. [PubMed: 9367155]
31. Simonet WS, Lacey DL, Dunstan CR, Kelley M, Chang MS, Luthy R, Nguyen HQ, Wooden S, Bennett L, Boone T, Shimamoto G, DeRose M, Elliott R, Colombero A, Tan HL, Trail G, Sullivan J, Davy E, Bucay N, Renshaw-Gegg L, Hughes TM, Hill D, Pattison W, Campbell P, Sander S, Van G, Tarpley J, Derby P, Lee R, Boyle WJ. Osteoprotegerin: A novel secreted protein involved in the regulation of bone density. Cell. 1997; 89:309–319. [PubMed: 9108485]
32. Lacey DL, Timms E, Tan HL, Kelley MJ, Dunstan CR, Burgess T, Elliott R, Colombero A, Elliott G, Scully S, Hsu H, Sullivan J, Hawkins N, Davy E, Capparelli C, Eli A, Qian YX, Kaufman S, Sarosi I, Shalhoub V, Senaldi G, Guo J, Delaney J, Boyle WJ. Osteoprotegerin ligand is a cytokine that regulates osteoclast differentiation and activation. Cell. 1998; 93:165–176. [PubMed: 9568710]
33. Yasuda H, Shima N, Nakagawa N, Yamaguchi K, Kinosaki M, Mochizuki S, Tomoyasu A, Yano K, Goto M, Murakami A, Tsuda E, Morinaga T, Higashio K, Udagawa N, Takahashi N, Suda T. Osteoclast differentiation factor is a ligand for osteoprotegerin/osteoclastogenesis-inhibitory factor and is identical to TRANCE/RANKL. Proc Natl Acad Sci U S A. 1998; 95:3597–3602. [PubMed: 9520411]

34. Takeshita S, Kaji K, Kudo A. Identification and characterization of the new osteoclast progenitor with macrophage phenotypes being able to differentiate into mature osteoclasts. J Bone Miner Res. 2000; 15:1477–1488. [PubMed: 10934646]

35. Chen EH, Grote E, Mohler W, Vignery A. Cell-cell fusion. FEBS Lett. 2007; 581:2181–2193. [PubMed: 17395182]

36. Staege H, Brauchlin A, Schoedon G, Schaffner A. Two novel genes FIND and LIND differentially expressed in deactivated and listeria-infected human macrophages. Immunogenetics. 2001; 53:105–113. [PubMed: 11345586]

37. Hartgers FC, Vissers JL, Looman MW, van Zoelen C, Huffine C, Figdor CG, Adema GJ. DC-STAMP, a novel multimembrane-spanning molecule preferentially expressed by dendritic cells. Eur J Immunol. 2000; 30:3585–3590. [PubMed: 11169400]

38. Yagi M, Miyamoto T, Sawatani Y, Iwamoto K, Hosogane N, Fujita N, Morita K, Ninomiya K, Suzuki T, Miyamoto K, Oike Y, Takeya M, Toyama Y, Suda T. DC-STAMP is essential for cell-cell fusion in osteoclasts and foreign body giant cells. J Exp Med. 2005; 202:345–351. [PubMed: 16061724]

39. Yagi M, Miyamoto T, Toyama Y, Suda T. Role of DC-STAMP in cellular fusion of osteoclasts and macrophage giant cells. J Bone Miner Metab. 2006; 24:355–358. [PubMed: 16937266]

40. Vignery A. Macrophage fusion: The making of osteoclasts and giant cells. J Exp Med. 2005; 202:337–340. [PubMed: 16061722]

41. Sawatani Y, Miyamoto T, Nagai S, Maruya M, Imai J, Miyamoto K, Fujita N, Ninomiya K, Suzuki T, Iwasaki R, Toyama Y, Shinohara M, Koyasu S, Suda T. The role of DC-STAMP in maintenance of immune tolerance through regulation of dendritic cell function. Int Immunol. 2008; 20:1259–1268. [PubMed: 18653699]

42. Kukita T, Wada N, Kukita A, Kakimoto T, Sandra F, Toh K, Nagata K, Iijima T, Horiuchi M, Matsusaki H, Hieshima K, Yoshie O, Nomiyama H. RANKL-induced DC-STAMP is essential for osteoclastogenesis. J Exp Med. 2004; 200:941–946. [PubMed: 15452179]

43. Miyamoto T. The dendritic cell-specific transmembrane protein DC-STAMP is essential for osteoclast fusion and osteoclast bone-resorbing activity. Mod Rheumatol. 2006; 16:341–342. [PubMed: 17164993]

44. Jones DH, Kong YY, Penninger JM. Role of RANKL and RANK in bone loss and arthritis. Ann Rheum Dis. 2002; 61(Suppl 2):ii32–9. [PubMed: 12379618]

45. Moreno JL, Kaczmarek M, Keegan AD, Tondravi M. IL-4 suppresses osteoclast development and mature osteoclast function by a STAT6-dependent mechanism: Irreversible inhibition of the differentiation program activated by RANKL. Blood. 2003; 102:1078–1086. [PubMed: 12689929]

46. Rivollier A, Mazzorana M, Tebib J, Piperno M, Aitsiselmi T, Rabourdin-Combe C, Jurdic P, Servet-Delprat C. Immature dendritic cell transdifferentiation into osteoclasts: A novel pathway sustained by the rheumatoid arthritis microenvironment. Blood. 2004; 104:4029–4037. [PubMed: 15308576]

47. Li P, Schwarz EM. The TNF-alpha transgenic mouse model of inflammatory arthritis. Springer Semin Immunopathol. 2003; 25:19–33. [PubMed: 12904889]

48. Abu-Amer Y, Ross FP, McHugh KP, Livolsi A, Peyron JF, Teitelbaum SL. Tumor necrosis factor-alpha activation of nuclear transcription factor-kappaB in marrow macrophages is mediated by c-src tyrosine phosphorylation of ikappa balpha. J Biol Chem. 1998; 273:29417–29423. [PubMed: 9792645]

49. Keffer J, Probert L, Cazlaris H, Georgopoulos S, Kaslaris E, Kioussis D, Kollias G. Transgenic mice expressing human tumour necrosis factor: A predictive genetic model of arthritis. EMBO J. 1991; 10:4025–4031. [PubMed: 1721867]

50. Diarra D, Stolina M, Polzer K, Zwerina J, Ominsky MS, Dwyer D, Korb A, Smolen J, Hoffmann M, Scheinecker C, van der Heide D, Landewe R, Lacey D, Richards WG, Schett G. Dickkopf-1 is a master regulator of joint remodeling. Nat Med. 2007; 13:156–163. [PubMed: 17237793]

51. Wong BR, Josien R, Choi Y. TRANCE is a TNF family member that regulates dendritic cell and osteoclast function. J Leukoc Biol. 1999; 65:715–724. [PubMed: 10380891]

52. Kong YY, Feige U, Sarosi I, Bolon B, Tafuri A, Morony S, Capparelli C, Li J, Elliott R, McCabe S, Wong T, Campagnuolo G, Moran E, Bogoch ER, Van G, Nguyen LT, Ohashi PS, Lacey DL,

Fish E, Boyle WJ, Penninger JM. Activated T cells regulate bone loss and joint destruction in adjuvant arthritis through osteoprotegerin ligand. Nature. 1999; 402:304–309. [PubMed: 10580503]

53. Abu-Amer Y. IL-4 abrogates osteoclastogenesis through STAT6-dependent inhibition of NF-kappaB. J Clin Invest. 2001; 107:1375–1385. [PubMed: 11390419]
54. Plows D, Kontogeorgos G, Kollias G. Mice lacking mature T and B lymphocytes develop arthritic lesions after immunization with type II collagen. J Immunol. 1999; 162:1018–1023. [PubMed: 9916728]
55. Harrington LE, Hatton RD, Mangan PR, Turner H, Murphy TL, Murphy KM, Weaver CT. Interleukin 17-producing CD4+ effector T cells develop via a lineage distinct from the T helper type 1 and 2 lineages. Nat Immunol. 2005; 6:1123–1132. [PubMed: 16200070]
56. Dong C. TH17 cells in development: An updated view of their molecular identity and genetic programming. Nat Rev Immunol. 2008; 8:337–348. [PubMed: 18408735]
57. Manel N, Unutmaz D, Littman DR. The differentiation of human T(H)-17 cells requires transforming growth factor-beta and induction of the nuclear receptor RORgammat. Nat Immunol. 2008; 9:641–649. [PubMed: 18454151]
58. Ouyang W, Kolls JK, Zheng Y. The biological functions of T helper 17 cell effector cytokines in inflammation. Immunity. 2008; 28:454–467. [PubMed: 18400188]
59. Paradowska A, Masliniski W, Grzybowska-Kowalczyk A, Lacki J. The function of interleukin 17 in the pathogenesis of rheumatoid arthritis. Arch Immunol Ther Exp (Warsz). 2007; 55:329–334. [PubMed: 18219763]
60. Lubberts E. The role of IL-17 and family members in the pathogenesis of arthritis. Curr Opin Investig Drugs. 2003; 4:572–577.
61. Bush KA, Farmer KM, Walker JS, Kirkham BW. Reduction of joint inflammation and bone erosion in rat adjuvant arthritis by treatment with interleukin-17 receptor IgG1 fc fusion protein. Arthritis Rheum. 2002; 46:802–805. [PubMed: 11920418]
62. Lubberts E, Koenders MI, Oppers-Walgreen B, van den Bersselaar L, Coenen-de Roo CJ, Joosten LA, van den Berg WB. Treatment with a neutralizing anti-murine interleukin-17 antibody after the onset of collagen-induced arthritis reduces joint inflammation, cartilage destruction, and bone erosion. Arthritis Rheum. 2004; 50:650–659. [PubMed: 14872510]
63. Chabaud M, Durand JM, Buchs N, Fossiez F, Page G, Frappart L, Miossec P. Human interleukin-17: A T cell-derived proinflammatory cytokine produced by the rheumatoid synovium. Arthritis Rheum. 1999; 42:963–970. [PubMed: 10323452]
64. Kotake S, Udagawa N, Takahashi N, Matsuzaki K, Itoh K, Ishiyama S, Saito S, Inoue K, Kamatani N, Gillespie MT, Martin TJ, Suda T. IL-17 in synovial fluids from patients with rheumatoid arthritis is a potent stimulator of osteoclastogenesis. J Clin Invest. 1999; 103:1345–1352. [PubMed: 10225978]
65. Shahrara S, Huang Q, Mandelin AM 2nd, Pope RM. TH-17 cells in rheumatoid arthritis. Arthritis Res Ther. 2008; 10:R93. [PubMed: 18710567]
66. Steiner G, Smolen J. Autoantibodies in rheumatoid arthritis and their clinical significance. Arthritis Res. 2002; 4 (Suppl 2):S1–5. [PubMed: 12110150]
67. Matthews R. Autoimmune diseases. the B cell slayer. Science. 2007; 318:1232–1233. [PubMed: 18033858]
68. Takemura S, Braun A, Crowson C, Kurtin PJ, Cofield RH, O'Fallon WM, Goronzy JJ, Weyand CM. Lymphoid neogenesis in rheumatoid synovitis. J Immunol. 2001; 167:1072–1080. [PubMed: 11441118]
69. Takemura S, Klimiuk PA, Braun A, Goronzy JJ, Weyand CM. T cell activation in rheumatoid synovium is B cell dependent. J Immunol. 2001; 167:4710–4718. [PubMed: 11591802]
70. Duddy ME, Alter A, Bar-Or A. Distinct profiles of human B cell effector cytokines: A role in immune regulation? J Immunol. 2004; 172:3422–3427. [PubMed: 15004141]
71. Li Y, Toraldo G, Li A, Yang X, Zhang H, Qian WP, Weitzmann MN. B cells and T cells are critical for the preservation of bone homeostasis and attainment of peak bone mass in vivo. Blood. 2007; 109:3839–3848. [PubMed: 17202317]

72. Gortz B, Hayer S, Redlich K, Zwerina J, Tohidast-Akrad M, Tuerk B, Hartmann C, Kollias G, Steiner G, Smolen JS, Schett G. Arthritis induces lymphocytic bone marrow inflammation and endosteal bone formation. J Bone Miner Res. 2004; 19:990–998. [PubMed: 15125796]
73. Mease P. Psoriatic arthritis update. Bull NYU Hosp Jt Dis. 2006; 64:25–31. [PubMed: 17121486]
74. van der Heijde D, Kavanaugh A, Gladman DD, Antoni C, Krueger GG, Guzzo C, Zhou B, Dooley LT, de Vlam K, Geusens P, Birbara C, Halter D, Beutler A. Infliximab inhibits progression of radiographic damage in patients with active psoriatic arthritis through one year of treatment: Results from the induction and maintenance psoriatic arthritis clinical trial 2. Arthritis Rheum. 2007; 56:2698–2707. [PubMed: 17665424]
75. Mease PJ, Gladman DD, Ritchlin CT, Ruderman EM, Steinfeld SD, Choy EH, Sharp JT, Ory PA, Perdok RJ, Weinberg MA. Adalimumab Effectiveness in Psoriatic Arthritis Trial Study Group: Adalimumab for the treatment of patients with moderately to severely active psoriatic arthritis: Results of a double-blind, randomized, placebo-controlled trial. Arthritis Rheum. 2005; 52:3279–3289. [PubMed: 16200601]
76. Edwards JCW, Szeczepanski L, Szechinski J. Efficacy and safety of rituximab, a B cell targeted chimeric antibody: a randomized placebo-controlled trial in rheumatoid arthritis [abstract]. Arthritis Rheum. 2002; 46:S446.
77. Cohen SB, Emery P, Greenwald MW, Dougados M, Furie RA, Genovese MC, Keystone EC, Loveless JE, Burmester GR, Cravets MW, Hessey EW, Shaw T, Totoritis MC. REFLEX Trial Group: Rituximab for rheumatoid arthritis refractory to anti-tumor necrosis factor therapy: Results of a multicenter, randomized, double-blind, placebo-controlled, phase III trial evaluating primary efficacy and safety at twenty-four weeks. Arthritis Rheum. 2006; 54:2793–2806. [PubMed: 16947627]
78. Manadan AM, Block JA. Rheumatoid arthritis: Beyond tumor necrosis factor-alpha antagonists, B cell depletion, and T cell blockade. Am J Ther. 2008; 15:53–58. [PubMed: 18223354]
79. Axmann R, Herman S, Zaiss M, Franz S, Polzer K, Zwerina J, Herrmann M, Smolen J, Schett G. CTLA-4 directly inhibits osteoclast formation. Ann Rheum Dis. 2008; 67:1603–1609. [PubMed: 18203760]
80. Schwarz EM, Ritchlin CT. Clinical development of anti-RANKL therapy. Arthritis Res Ther. 2007; 9 (Suppl 1):S7. [PubMed: 17634146]
81. Bekker PJ, Holloway D, Nakanishi A, Arrighi M, Leese PT, Dunstan CR. The effect of a single dose of osteoprotegerin in postmenopausal women. J Bone Miner Res. 2001; 16:348–360. [PubMed: 11204435]
82. McClung MR, Lewiecki EM, Cohen SB, Bolognese MA, Woodson GC, Moffett AH, Peacock M, Miller PD, Lederman SN, Chesnut CH, Lain D, Kivitz AJ, Holloway DL, Zhang C, Peterson MC, Bekker PJ. AMG 162 Bone Loss Study Group: Denosumab in postmenopausal women with low bone mineral density. N Engl J Med. 2006; 354:821–831. [PubMed: 16495394]