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Immunology of Gut Bone Signaling

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Abstract

In recent years a link between the gastro-intestinal tract and bone health has started to gain significant attention. Dysbiosis of the intestinal microbiota has been linked to the pathology of a number of diseases which are associated with bone loss. In addition modulation of the intestinal microbiota with probiotic bacteria has revealed to have both beneficial local and systemic effects. In the present chapter we discuss the intestinal and bone immune systems, explore how intestinal disease affects the immune system and examine how these pathologic changes could adversely impact bone health.

The Digestive Tract

The gastrointestinal tract forms a continuous tube from the mouth to the anus with the intestine consisting of two major segments: the small and large intestines. Each intestinal segment consists of specialized regions that mediate nutrient digestion and absorption (small intestine), control water and electrolyte absorption (small and large intestine), absorb vitamin B12 and bile absorption (ileum), regulate the movement of digested products/chyme through the tract, as well as interact with and influence the intestinal microbiome (Fig 1). Partly due to this constant interaction with intestinal flora, the digestive tract has one of the largest immune compartments in the body (1). By providing the body with the ability to sense the environment (dietary composition, pathogens, etc...) and to absorb water and nutrients needed to maintain homeostasis, the gut is a crucial organ for survival.

Descending from the stomach, the human small intestine is approximately 5 meters in length and has three sections (based on histology and function): the duodenum, jejunum, and ileum. The small intestine is characterized by plicae circulares (folds in the duodenum), villi which are finger like projections that extend out into the lumen throughout the small intestine, and microvilli at the apical surface of epithelial cells; together these structures vastly increase the surface area for nutrient absorption; while once thought to be greater than the area of a

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tennis court, more recent studies indicate the surface area is more on the order of half a badminton court (32 meters) (2). The large intestine consists of the cecum; ascending, transverse and descending colon; rectum and ends in the anus. While shorter than the small intestine, approximately 1.5 m, the large intestine is wider in diameter (4.8 cm vs 2.5 cm diameter in the small intestine). In contrast to the small intestine, the large intestine does not contain villi (1).

Though different in gross morphology due to their divergent functions, all sections of the intestinal tract are comprised of four layers visualized cross-sectionally: the mucosa, submucosa, muscularis externa and the serosa. The mucosa is vital for the regulation of many immunological processes aiding in the regulation and appropriate tolerance of the microbiota. It is made up of three sub-layers: 1) the epithelial layer which contains strong cell:cell connections (tight junctions) and forms a strong barrier that has low permeability, 2) the underlying lamina propria where connective tissue, blood vessels and immune cells are located, and 3) the muscularis mucosae a thin muscular layer under the lamina propria (Fig 2). The mucosa plays a key role in the intestinal immunological defenses ensuring that luminal pathogens do not cross the intestinal barrier (1). In this chapter we will focus on gut and bone immunology separately and then discuss how the immune system can serve as a link between the two organs.

The Intestinal Immune System

The adult human intestine is residence to 10^{13} to 10^{14} microorganisms (3,4). Gut microbiota, dietary proteins, and pathogens are recognized by the immune system as non-self, and therefore can elicit an immune response. The gut must both protect the host from invading pathogens while maintaining immune tolerance against beneficial dietary protein and harmless commensal microbes (5–8). Given that immune surveillance is critical, it is not surprising that the intestinal tract contains the largest number of immune cells of any organ in the body, known as the gut-associated lymphoid tissue (GALT). In addition, the intestine has specialized epithelial cells (Paneth and goblet cells) that secrete anti-bacterial factors, and has intraepithelial lymphocytes to sample luminal antigens (Fig 3). The intestine also contains organized lymphoid structures comprised of Peyer's patches, solitary isolated lymphoid tissues, lamina propria and draining lymph nodes. In the following section we will discuss each of these barrier and surveillance components and how they influence gut defense.

Paneth Cells

Paneth cells are specialized, long-lived cells that migrate to the base of crypts after differentiating from stem cells. They produce anti-microbial peptides including; regenerating islet-derived protein III γ (REGIII γ), defensins and lysozymes, which are secreted into crypt/lumen area to reduce bacterial survival near the epithelial barrier. Paneth cells are only found in the small intestine with the highest concentration observed in the ileum. They play a key role in small intestinal homeostasis. Dysregulation in their function results in an increased risk of developing adverse conditions such as Crohn's disease (9).

Goblet Cells

Goblet cells are responsible for the production and secretion of mucus. The frequency of goblet cells increases along the gastrointestinal tract; comprising ~ 10% of epithelial cells in the upper small intestine and ~25% of epithelial cells in the distal colon. The mucus layer produced by goblet cells covers the mucosa/epithelial cells and forms a barrier to luminal toxins and bacteria. In the small intestine the secreted mucus exists as a single layer and is loosely adherent. In contrast, the mucus layer in the colon is divided into two sections: a loose outer layer that contains bacteria and a dense bacteria-free inner layer that is firmly attached to the epithelium (10–12). In addition to being a physical barrier, mucus contains glycol proteins, IgA, antimicrobial peptides and other substances that are toxic to many bacteria (1,13).

Intraepithelial Lymphocytes

The majority of intraepithelial lymphocytes (IELs) are antigen-experienced T-lymphocytes belonging to both the T cell receptor- $\gamma\delta$ (TCR $\gamma\delta$) and TCR $\alpha\beta$ lineages and form a frontline of immune defense against invading pathogens (14). IELs are heterogeneous with alternate distribution patterns in the small and large intestine based upon the physiological conditions. TCR $\gamma\delta$ T cells make up approximately 60% of IELs in the small intestine. The majority of IELs can express effector cytokines including, interleukin (IL)-2, IL-4, IL-17 and interferon gamma (IFN γ) (14,15). Under normal physiological conditions IELs display regulatory functions and suppress excessive inflammation that can damage the delicate single cell layer epithelial barrier. Thus their primary role is to ensure the integrity of the intestinal epithelium and maintain local immune quiescence (16,17). However, in some pathological conditions excessive or uncontrolled IEL cytotoxicity can promote inflammation which may initiate or exacerbate inflammatory bowel diseases or celiac disease (14,18).

Peyer's Patches

Peyer's patches are the best characterized lymphoid structures in the GALT (Fig 4). Peyer's patches are macroscopic structures that are visible on the anti-mesenteric side of the small intestine. The size and number of Peyer's patches increases from the jejunum to the ileum with the highest concentration found in the distal ileum; Peyer's patches are rarely observed in the duodenum (19). Peyer's patches are characterized by an overlying follicle-associated epithelium. The follicle-associated epithelium contains microfold cells (M cells) that are specialized for the uptake and transport of antigens from the lumen into Peyer's patches, where they are taken up by dendritic cells and presented to the lymphocytes for adaptive immune response (20). Peyer's patches are composed of germinal centers with B cell lymphoid follicles that are flanked by smaller T cell areas (21). While the large intestine does not have Peyer's patches *per se*, equivalent macroscopic M cell-containing structures can be found; around the ileocaecal valve these structures are known as caecal patches while throughout the colon and rectum these structures are known as colonic patches (22).

Solitary Isolated Lymphoid Tissues

Solitary isolated lymphoid tissues (SILTs) can only be detected microscopically and range in size from small cryopatches to larger mature isolated lymphoid follicles (24). In contrast to

Peyer's patches, the isolated lymphoid follicles consist of B cells with no clear T cell zone but do contain germinal centers (25). SILTs increase in number down the human small intestine; with 10-fold more in the distal ileum compared to the proximal jejunum. In the colon numbers of SILTs triple from the ascending colon to the recto-sigmoid colon (24). The role of SILTs is relatively unknown when compared to the larger lymphoid tissues however; studies have suggested that they may have a similar role to Peyer's patches in the induction of an immune response (26,27)

Lamina Propria

The lamina propria is comprised of loosely packed connective tissue forming the supportive structure for the villi and also contains the blood supply, lymph drainage and nervous system for the mucosa (1). In addition, the lamina propria contains many cells of the innate and adaptive immune system: dendritic cells (DCs), macrophages, eosinophils, mast cells, B cells and T cells (Fig 5). CD4⁺ and CD8⁺ T cells are found in a ratio of approximately 2:1 with the majority displaying an effector memory phenotype (28). The CD4⁺ population of T cells in the lamina propria is very diverse with T helper (Th) 1, Th2, Th17, forkhead box P3-positive (Foxp3) regulatory T cells (T_{reg}) and Foxp3-negative (T_R1) subsets present. Th17 cells decrease in number from the duodenum to the colon whereas T_{reg} numbers are highest in the colon. Numbers of Th1 and Th2 cells have been reported to be consistent along the length of the intestine (29).

The lamina propria also contains large numbers of B cells and plasma cells, with the highest density at the proximal and distal ends of the intestinal tract (30). IgA plasma cells are the most abundant throughout the intestine; with the frequency increasing from approximately 75% of plasma cells in the duodenum to 90% in the colon. The majority of the remaining plasma cells secrete IgM (1).

The most abundant leukocyte in the healthy lamina propria is the macrophage which is present in the small and large intestine. Intestinal macrophages are involved in maintaining intestinal homeostasis; phagocytosing and degrading microorganisms and dead cells while releasing factors that stimulate renewal of the epithelium. Lamina propria macrophages produce large quantities of IL-10; an anti-inflammatory cytokine that promotes the survival and function of Foxp3⁺ T_{reg} cells (31,32).

Intestinal DCs are characterized by the expression of the markers CD11c⁺, MHC II⁺ and the absence of CD64 and F4/80. Four main subsets of DCs have been described in the mouse lamina propria and are classified on the basis of their expression of CD103 and CD11b (33). These subsets have been suggested to have distinct roles in intestinal immune homeostasis. DCs continuously migrate from the gut to the draining lymph nodes a process required for tolerance induction to 'self' or food antigens (34). Following antigen stimulation DCs can activate T cells to induce the appropriate inflammatory response, promote T cell differentiation into effector or regulatory subsets, induce B cell isotype switching as well as mucosal homing properties to T and B cells (34).

Mast cells are present throughout the healthy intestine and account for 2-3% of lamina propria cells under normal conditions (35). They produce factors that mediate epithelial

barrier integrity, peristalsis, vascular tone and permeability (1). Furthermore, mast cells express toll-like receptors suggesting that these cells can also detect microorganisms and/or the associated structures (35). Eosinophils are believed to have a role in tissue repair in both physiological and pathological conditions in both the small and large intestines. In addition, studies have suggested that eosinophils may have a role in maintaining IgA⁺ plasma cell numbers, DCs and Foxp3⁺ T_{reg} cells in the lamina propria (36).

Draining Lymph Nodes

The intestinal lymph nodes are the largest in the body. In rodents, different segments of the intestine drain to specific nodes; the duodenum primarily drains to a small node located in the pancreatic tissue; the jejunum drains to the middle mesenteric lymph node (MLN); the distal ileum, caecum and ascending colon drain to the distal MLN; the traverse colon drains to two small nodes buried in the pancreas; and the descending colon and rectum drain to the caudal lymph node. Similar region specific differences in lymph drainage are also observed in humans (1). The intestinal lymphatic system plays a key role in acquired immunity and tolerance; recirculating lymphocytes which pass from the blood to tissue then to the lymph nodes before returning to the circulation (37). Studies have revealed that the MLNs are key sites for the induction of tolerance to food and commensal bacteria antigens. Antigen-laden DCs migrate from the intestinal epithelial barrier to the MLNs where they can “educate” MLN resident T cells. In addition, the MLNs act as a ‘firewall’ stopping commensal bacteria-laden DCs from entering the thoracic duct lymph system and reaching the systemic circulation. Bacteria that may be released by DCs upon cell death are eliminated by MLN macrophages (38).

Inflammatory Intestinal Pathologies

Dysregulation of the intestinal immune system is involved in a number of serious pathological conditions including: celiac disease, Crohn's disease and ulcerative colitis. While celiac disease is localized to the duodenum, Crohn's disease is most common in the distal small intestine and ulcerative colitis in the colon (39,40). The specific cytokines dysregulated in the two major forms of inflammatory bowel disease, Crohn's disease and ulcerative colitis, are also different. Crohn's disease is driven by a T_H1 / T_H17 response in which IL-12, IL-23, IL-17 and IFN γ play key roles while ulcerative colitis is driven by a T_H2-like response in which IL-5 and IL-13 play a key role (41,42). Interestingly, these intestinal pathologies are also associated with bone pathology with a significant decrease in trabecular bone leading to the development of osteoporosis (43,44).

Osteoimmunology – The Bone Immune System

Bone remodeling is a coordinated process that maintains the rate of osteoblast (OB) bone formation and osteoclast (OC) bone resorption, ensuring the maintenance of skeletal integrity. Under normal physiological conditions the osteoblast and osteoclast cross-talk, with each cell able to regulate the differentiation and action of the other. The process of canonical osteoclast differentiation is regulated by the action of three critical cytokines released by the osteoblast: macrophage colony stimulating factor (MCSF), receptor activator of nuclear-factor kappa B ligand (RANKL) and the soluble RANKL receptor

osteoprotegerin (OPG) (45). Conversely, the osteoclast can modulate osteoblast differentiation through the secretion of coupling factors including BMP-6 and Wnt10b (46,47) and membrane-bound components including ephrin/Eph family members (47) (Fig 6). In addition to the crosstalk between bone cells, the immune system has been demonstrated to play a significant role in regulating bone remodeling. This section will discuss the role of the immune system in the regulation of bone cell activity.

Immune Cells and Bone

T-lymphocyte regulation of bone

As discussed before, T cells are classed into two main categories based on the subunits of the T cell receptor (TCR) they express; $\alpha\beta$ or $\gamma\delta$. The majority of T cells express the $\alpha\beta$ subunits and can be further divided based on the expression of the CD4 and CD8 markers (48). The CD4⁺ T cells can additionally be sub-divided based on their cytokine profiles: T_h1, T_h2, T_{reg} and T_h17. While T lymphocytes are critical mediators of the adaptive immune response they are also capable of modulating bone remodeling.

Under physiological conditions T cells are not major contributors to the levels of RANKL because T cell-deficient nude mice do not exhibit diminished RANKL mRNA in their BM (49). However, resting T cells are demonstrated to have a bone protective role, stimulating B-lymphocyte-mediated OPG production through CD40 – CD40L interaction (49). Consistent with this, T cell-deficient nude mouse have baseline OC numbers and reduced bone density compared to controls (49,50). In addition, CD4/CD8 T cell depleted mice exhibit significantly elevated *in vitro* osteoclastogenesis (51). In contrast, activation of T cells by infection and inflammation leads to an increase in T cell-derived osteoclastogenic factors such as RANKL and tumor necrosis factor alpha (TNF α), subsequently resulting in increased bone resorption as seen in conditions such as osteoporosis, the inflammatory arthritides and periodontitis (52). It should be noted nonetheless, that distinct T cell subsets have differing functions when it comes to regulating bone turnover (Fig 7).

CD4⁺ T cell subsets and their effects on bone

Following antigen stimulation naïve CD4 T cells proliferate and undergo differentiation into distinct effector subsets that are classified according to their cytokine profiles. T helper 1 (T_h1) cells are characterized by the expression of TNF α , interferon gamma (IFN γ) and interleukin (IL)-2; T_h2 cells produce IL-4, IL-5, IL-6, IL-9, IL-10 and IL-13; Th17 cells produce IL-17A, IL-17F, IL-21 and IL-22; and T_{regs} express CD25 and Foxp3 and produce IL-10 and TGF β (53,54). Of these subsets T_h1, T_h2 and T_{reg} cells suppress osteoclast formation while T_h17 cells stimulate osteoclastogenesis. Inhibition by Th1 and Th2 is in part dependent on the number of T cells as well as on the expression of IFN γ and IL4 (55).

T_{regs} can modulate osteoclast formation via direct cell-to-cell contact as well as through cytokine production (56). Mechanism of direct cell contact is through cytotoxic T-lymphocyte antigen 4 (CTLA-4) expressed on Tregs (56). Furthermore, addition of CTLA-4 to osteoclast precursor cells dose-dependently suppresses osteoclast formation, a process reversed by anti-CTLA-4 antibodies (56). These data suggest that CTLA-4 may directly

suppress osteoclastogenesis by binding to CD80/CD86 on mononuclear OC precursors (57). In addition to the direct suppressive effect of T_{regs} on osteoclast differentiation, expression of TGFβ, IL-4 and IL-10 were observed to contribute to the inhibitory effect on osteoclastogenesis (56). The role of these cytokines in the mechanism by which T_{regs} inhibit osteoclast formation is supported in studies by Luo et al (58) and Kim et al (59) who demonstrated that CD4 T_{regs} suppressed osteoclast formation from peripheral blood mononuclear cells (PBMCs) via the expression of the cytokines TGFβ, IL-4 and IL-10.

Compared to the above T helper subsets, T_H17 cells primarily support osteoclast differentiation through the production of IL-17A and are believed to play a crucial role in inflammation and the development of autoimmune diseases such as rheumatoid arthritis. Furthermore, T_H17 cells express RANKL and TNFα which can directly act on osteoclast precursors to induce osteoclastogenesis (55,60,61)

CD8⁺ T lymphocyte regulation of bone

Activated CD8⁺ T cells express high levels of RANKL and OPG. When cultured with osteoclast precursors, CD8⁺ T cells however, inhibit soluble RANKL-induced osteoclast formation. Interestingly, blocking OPG with an antibody does not reverse the inhibitory effect of the CD8⁺ T cells suggesting the involvement of other factors (62). The bone protective role of CD8⁺ T cells has also been reported by John et al (63) who revealed that depletion of CD8⁺ T cells from murine bone marrow increases osteoclast differentiation.

CD8⁺ T cells also play a key role in the anabolic effect of parathyroid hormone (PTH). Treatment of mice with intermittent PTH (iPTH) significantly increases the production of Wnt10b by bone marrow CD8⁺ T cells subsequently activating canonical Wnt signaling in osteoblasts (64). CD8⁺ T-cell-dependent anabolic effect of PTH was demonstrated in T-cell-null mice which displayed diminished Wnt signaling in pre-osteoblasts and blunted osteoblastic commitment, proliferation, differentiation, and life span (64). In addition, conditional silencing of PTH receptor 1 in T cells blunted the ability of iPTH to induce T cell Wnt10b production, activate osteoblastic Wnt signaling and increased bone turnover (65).

CD8⁺ T Regulatory cells in bone health

In contrast to CD4⁺ T_{regs}, CD8⁺ T_{regs} have not been extensively studied partly due to their low abundance in lymphoid tissues (57); though like their CD4 counterparts they express both CD25 and the transcription factor Foxp3 (66). *In vitro*, the osteoclast-induced Foxp3⁺ CD8⁺ T_{reg} cells potently and directly suppressed osteoclast differentiation and resorptive activity, but did not affect mature osteoclast survival. This suppressive effect of Foxp3⁺ CD8⁺ T_{regs} was relieved following antibody blockade against IL-6, IL-10 and IFNγ (67). Using adoptive transfer experiments Foxp3⁺ CD8⁺ T_{regs} were shown to limit the bone loss induced by RANKL administration as well as protect against ovariectomy-induced bone loss (68).

B-lymphocytes and their regulation of bone cells

B cells have a close and multifaceted relationship with bone cells (69). B cells are differentiated from hematopoietic stem cells (HSCs) in niches found on the endosteal bone surface (57,69). These niches are supported by osteoblasts which sustain the HSCs and B cell differentiation (69). The role of B cells in normal bone remodeling is not fully clear however, cells of the B-cell-lineage have been demonstrated to be responsible for 64% of total BM OPG production, with 45% derived from mature B cells (49). In support of a role for B cells in physiological bone remodeling B cell knockout (KO) mice have an osteoporotic phenotype, due to elevated osteoclast bone resorption and decreased BM OPG expression; a phenotype rescued by B cell reconstitution (49). In addition to a role in physiological bone remodeling, activated B cells have been implicated in many inflammatory diseases associated with osteoporosis; such as estrogen deficiency, multiple myeloma and periodontal disease (62,70).

In conditions of estrogen depletion B lymphogenesis is increased. Consistent with this, estrogen treatment downregulates B lymphogenesis (71,72). Furthermore, B cells isolated from the bone marrow of post-menopausal women have also been shown to express RANKL (73) suggesting a role for B cells in menopausal bone loss. This is further supported in a study by Onal et al (74) using B cell specific RANKL KO mice. These KO mice were partially protected from ovariectomy-induced bone loss. This protection occurred in the trabecular bone, but not the cortical bone, and was associated with a failure to increase osteoclast numbers. Conversely however, in an *in vitro* model of osteoclastogenesis, B cells isolated from the peripheral blood inhibited OC formation via the secretion of TGF β , a cytokine that induces OC apoptosis (75); suggesting that the role of B cells in estrogen-deficiency induced bone loss may not be clear-cut.

In multiple myeloma B cells and B cell-derived plasma cells have been reported to support osteoclastogenesis directly through expression of RANKL and indirectly through expression of IL-7 (76,77). In addition, malignant B-cell-derived plasma cells have been shown to secrete cytokines such as sclerostin and DKK1 which inhibit osteoblastogenesis (77,78); affecting the balance of remodeling on two fronts. In human chronic periodontitis B-lineage cells (B lymphocytes and plasma cells) have been revealed to dominate the inflammatory infiltrate suggesting that these cells play a key role in the associated bone loss (79). This is supported by murine studies in the ligature-induced periodontitis model and the *P. gingivalis* oral infection model, where reduced bone loss was observed in B cell-deficient mice (μ MT) compared to wild-type controls (80,81). Furthermore, antibody neutralization of a proliferation-inducing ligand (APRIL) or B-lymphocyte stimulator (BLys), two cytokines important for the maturation and survival of B cells, diminished the number of B cells in the gingival tissue and inhibited bone loss in wild-type but not in B cell- deficient mice (80).

Regulation of bone by Neutrophils

Neutrophils are implicated with bone loss associated with murine models of inflammatory arthritis (82–84) and human periodontitis (85). Human and murine neutrophils strongly upregulate their expression of RANKL following LPS treatment, stimulating osteoclastic bone resorption through direct cell-cell contact with osteoclasts (86) (Fig 8).

Role of Dendritic Cells in bone health

Dendritic cells (DCs) are broadly composed of two main subsets: conventional (cDC) and plasmacytoid (pDC). The main function of cDCs is to process and present antigens to antigen-specific naïve T cells whereas the function of pDCs is the secretion of IFN α / β in response to viral infection (87). While DCs do not appear to play a role in physiological bone remodeling in chronic inflammatory conditions, such as RA, immature cDCs have the potential to serve as osteoclast precursors (88,89). These DC-derived osteoclasts are functionally active and able to resorb bone (90,91) (Fig 8).

Immune Cytokines and Bone

TNF α

TNF α can affect bone remodeling in multiple ways, acting on the osteoclast and the osteoblast (92). TNF α increases osteoclast formation by upregulating osteoblast expression of RANKL and MCSF (93,94). Furthermore, TNF α can act on osteoclast precursors in permissive levels of RANKL inducing their differentiation by enhancing its effects (95–97). Blockade of TNF activity in bone pathological conditions such as inflammatory arthritis and estrogen-deficiency have been shown to protect against the associated bone loss (98,99). In addition to stimulating osteoclast formation and bone resorption TNF α also inhibits osteoblast proliferation, differentiation and matrix deposition (100,101). Moreover, TNF α is pro-apoptotic for osteoblasts, potentially through Fas – FasL signaling (102,103), though the significance of this pathway in osteoblast apoptosis has been disputed in recent studies (104). Interestingly however, low levels of TNF have been revealed to induce osteogenic differentiation via upregulation of the transcription factors Runx2 and Osx (92,105) demonstrating a complex role for TNF α in regulation of bone remodeling.

Other TNF super family members

TNF-like protein 1A (TL1A) has been demonstrated to enhance RANKL induced osteoclastogenesis in murine and human models (106,107), furthermore TL1A has been linked to the adverse bone pathogenesis observed in inflammatory arthritis (106–108). Studies have also suggested that TL1A, signaling through death receptor 3 (DR3), may affect osteoblast differentiation and mineralization (109).

LIGHT (homologous to lymphotoxins exhibiting inducible expression and competing with herpes simplex virus glycoprotein D for herpesvirus entry mediator [HVEM], a receptor expressed by T lymphocytes) is elevated in conditions such as RA and multiple myeloma (110,111). In vitro, LIGHT has been reported to induce osteoclastogenesis in RANKL-dependent and –independent manners and indirectly inhibit osteoblastogenesis (110,111).

TNF-related apoptosis inducing ligand (TRAIL) is another member of the TNFSF that has biphasic effects on bone (112). TRAIL has been shown to induce apoptosis of osteoclasts but not osteoblasts (113,114). In addition, *in vivo*, injection of TRAIL for 8 days in 4-wk-old mice induces an increase in bone mass (115).

Interleukin-1

IL-1 plays an important role in pathological bone loss as seen in conditions such as RA and estrogen-deficiency. In murine models of menopause (ovariectomy) mice deficient in IL-1 signaling are protected against bone loss while treatment with IL-1 receptor antagonists reduces osteoclast formation and activity (116,117). In the collagen-induced arthritis model anti-IL-1 β and anti-IL-1R treatment reduced arthritis severity and protected against focal bone erosions (118). IL-1 acts on the bone cells in a number of ways (119); IL-1 enhances RANKL expression from osteoblasts / stromal cells (93); indirectly stimulates osteoclast differentiation by stimulating prostaglandin E₂ synthesis in osteoblasts (120); induces osteoclast precursor fusion into the multinucleated osteoclast (121); enhances osteoclast resorptive activity (121); and directly effects the survival of osteoclasts (122,123).

Interleukin-6

The role of IL-6 in osteoclast bone resorption is currently debated with studies revealing that it can both stimulate bone resorption, inhibit osteoclast differentiation or has no effect depending on the model used (124–126). However, in menopausal women, levels of IL-6 are elevated while in the murine OVX model IL-6 has been linked to increased osteoclast development (127). The role of IL-6 in estrogen-deficiency induced bone loss is further supported in the IL-6 knockout mouse which is protected against development of osteoporosis (128). A role for IL-6 in bone loss has also been reported in diseases such as Paget's disease and rheumatoid arthritis (129,130).

Interleukin-7

IL-7 is an osteoclastogenic cytokine, produced in the bone marrow primarily by stromal cells and osteoblasts, which is able to stimulate B and T cells (131). IL-7 promotes osteoclastogenesis by upregulating T and B cell-derived RANKL and TNF α (50,132). In the OVX model, increased production of IL-7 has been postulated to be a key factor in the uncoupling of bone formation from bone resorption (131).

Interleukin-10

IL-10, an anti-inflammatory cytokine, is mainly expressed by activated T and B cells. IL-10 has potent inhibitory effects on osteoclastogenesis both direct and indirect. IL-10 directly suppresses osteoclast formation by reducing nuclear factor of activated T cells, cytoplasmic 1 (NFATc1) expression and its translocation to the nucleus (133) and by suppressing c-Fos and c-Jun (134). Indirectly IL-10 has been shown to modulate osteoclast formation by increasing gene expression of OPG and decreasing RANKL gene expression in dental follicle cells (135).

Interleukin-17A

IL-17A acts on osteoclasts via both direct and indirect mechanisms. In an *in vitro* system IL-17A has been shown to directly act on human osteoclast precursors upregulating expression of RANK and rendering the cells more sensitive to the actions of RANKL (136). In addition, IL-17A elevates stromal cell expression of RANKL and synovial macrophage expression of the osteoclastogenic factors TNF α and IL-1 β , indirectly effecting

osteoclastogenesis (137). Conversely, IL-17A has been demonstrated to exhibit strong osteogenic effects when exposed directly to mesenchymal stem cells (MSCs) acting synergistically with bone morphogenetic protein 2 (BMP-2) (138).

Interferon γ

The effect of IFN γ on osteoclastogenesis is controversial. *In vitro*, IFN γ has been reported to inhibit osteoclastogenesis (139,140) and downregulate expression of cathepsin K (141). Conversely, IFN γ has also been reported to indirectly stimulate osteoclast resorptive activity by enhancing RANKL and TNF α production by T cells (140). *In vivo*, treatment of T cell-deficient nude mice with recombinant IFN γ had no effect on bone density however, adoptive transfer of wildtype T cells into these mice resulted in a significant decrease in vertebral bone mineral density (140).

TGF β

TGF β has a complex role in bone remodeling and is thought to have a key contribution in the coupling of the osteoblasts and osteoclasts. TGF β is expressed in a latent form by numerous cell types, including osteoblasts, stromal cells and bone marrow cells that must be activated to exert its effect (57). TGF β has been reported to stimulate osteoclastogenesis (142–144) and induce osteoclast apoptosis (145,146). Likewise in osteoblasts, TGF β has been reported to suppress differentiation (147), inhibit mineralization (148), induce osteoblast precursor migration and proliferation (149) and block apoptosis (150).

Immune Involvement and Gut-Bone Signaling in Pathophysiology

Celiac Disease – immune system and bone responses

Celiac disease is an enteric autoimmune disorder that is triggered by ingestion of gluten; a protein present in wheat, rye, and barley (44). Some of the typical gastrointestinal (GI) symptoms of the disease include diarrhea, abdominal pain, distention, weight loss, and failure to thrive in children. Other symptoms may also be present with or without GI symptoms, including anemia, low bone mineral density, elevated liver enzymes, unexpected weight loss, prolonged fatigue and infertility (44).

Celiac disease can severely affect bone health in patients, causing metabolic bone diseases such as low bone mass, osteoporosis, secondary hyperparathyroidism, and osteomalacia (44). Multiple mechanisms can be attributed to the development of these bone disorders. Mucosal atrophy in the intestinal lumen due to celiac disease impairs calcium absorption. This causes a significant increase in parathyroid hormone levels that stimulates bone degradation by osteoclasts in an effort to avoid hypocalcemia. Calcium loss from the bones can lead to osteopenia and osteoporosis, increasing the fracture risk. Celiac disease can also cause hypogonadism, which can subsequently have an adverse effect on the bone (44).

Inflammation from celiac disease is characterized by villus atrophy, crypt cell hyperplasia, and infiltration of T-lymphocytes in epithelial and lamina propria compartments (151). The recruited active T cells produce high levels of pro-inflammatory cytokines that can eventually damage intestinal tissue (151). Studies have revealed that celiac disease is

associated with exacerbated production of Th1 cytokines (eg. IFN γ and IL-21). In addition, expression of IL-17A, IL-1 β , IL-6, IL-8 and IL-15 have been shown to be upregulated in patients with active celiac disease. TNF α and IL-6 are elevated however, in refractory celiac disease (not responsive to a gluten free diet) (151). This elevation in serum pro-inflammatory cytokines observed in celiac disease could directly influence bone remodeling by stimulating osteoclastogenesis or indirectly by increasing the RANKL / OPG ratio (44,152,153). The area most affected by bone remodeling in celiac disease, is the trabecular bone, which is the most metabolically active bone section (44). Increased bone resorption in patients with celiac disease leads to thinner and fewer trabeculae thus resulting in increased fracture risk (44).

Inflammatory Bowel Disease –intestinal immune responses

Inflammatory bowel disease (IBD) is characterized by chronic recurrent inflammation of all or part of the gastrointestinal (GI) tract. The most common types of IBD are Crohn's Disease (CD) and Ulcerative Colitis (UC). Crohn's disease can affect the GI tract anywhere from the mouth to the anus, with the most common area affected being the ileum. Ulcerative colitis however, is specific to the colon (154,155). In 2014 IBD was reported to affect 1.6 million Americans and as many as 70,000 new cases are reported per year (CCFA). The immune system plays a critical role in the pathogenesis of IBD, with the adaptive immune system thought to be one of the main contributors to IBD pathogenesis (156). Pro-inflammatory cytokine producing T-helper cells are thought to be over-active whereas, anti-inflammatory T_{regs} are ineffective at resolving the inflammation (156).

At the onset of IBD, weakening of the epithelial barrier promotes increased permeability resulting in increased contact between the intestinal microbiota and the mucosal immune system. This excessive contact causes massive influx of neutrophils as well as loss of tolerance by activating dendritic cells (157). In addition to neutrophils, IBD pathology is also associated with an influx of lymphocytes into the mucosa (156). These cells produce vast quantities of pro-inflammatory cytokines (41,42,158). Increased activation especially of the IL-1 pathway is more active during the onset of inflammation rather than during the sustained phase, especially in the colon (42). Members of the IL-12 family of heterodimeric cytokines including IL-12, IL-23, IL-27, and IL-35 have been shown to be upregulated in antigen presenting cells (42,158), while TNF α has been shown to be enhanced in mononuclear cells. The effects of TNF are suggested to be two-fold; promoting inflammation by binding to its receptors, as well as causing cell death through activation of receptor-interacting protein kinase 1 and caspase 3 proteins (42,158).

Among the many immune cell types involved in the pathogenesis of IBD, T lymphocytes are thought to be critical (42). Depending on the type of IBD, involvement of the specific cytokine also differs. In Crohn's Disease, Th1-mediated increases in IFN γ and IL-2 are thought to be important. In contrast, in Ulcerative Colitis Th2-mediated IL-5 and IL-13 have been shown to be critical in the pathogenesis. However, in UC non-classical natural killer T cells have also been identified to have an atypical cytokine response (42). This response has been linked to fibrosis as well as altered tight junction function and apoptosis of intestinal

epithelial cells (IECs), driving intestinal epithelial cell ulceration. This response from the NKT cells has been found to be mainly mediated through IL-13 (42).

Regulatory T cells (T_{regs}) ($CD4^+CD25^+FoxP3^+$) have been shown to have a protective role in colitis; through expression of the anti-inflammatory cytokines IL-10 and transforming growth factor beta ($TGF\beta$) (42). In CD and UC patients T_{reg} numbers are increased in the inflamed lamina propria compared to uninflamed mucosa and healthy controls. Why these cells fail to control the inflammation is currently unknown however, it has been suggested that effector T cells are resistant to the effects of inhibitory cytokines such as $TGF-\beta$ in the context of inflammation (40).

Inflammatory Bowel Disease – bone immune responses

IBD is associated with significant and divergent responses in lymphoid and myeloid hematopoiesis in the bone marrow. In a study by Trottier et al (159) the dextran sodium sulfate (DSS)-induced model of colitis resulted in a 60% and 80% increase in monocytic and granulocytic lineages, respectively; consistent with the massive influx of monocytes and neutrophils into the gut. In contrast, the early lineages of B and T cells declined in the marrow and thymus. Furthermore, a significant 40% decrease was also observed in cells of erythrocytic lineage. Because bone marrow is present within the bone structure, it is conceivable that intestinal inflammation such as what is seen in IBD can have profound influences not only on bone marrow composition but also on bone cells themselves and this is aided by changes in and influence of inflammatory cytokines. Previous studies in animal models of IBD demonstrate that inflammatory cytokines such as $TNF\alpha$, IL-1 α , IL-1 β , IL-6, IL-11, and IL-17A are linked to decreased bone volume, bone formation rates, and osteoid surface (154,160–164). In some studies, osteoclast surface is increased which further exacerbates bone loss (160). Patients with IBD have elevated levels of pro-inflammatory osteoclastogenic cytokines including; $TNF\alpha$, IL-1, IL-6, IL-11, IL-17, $TGF\alpha$, epidermal growth factor, and prostaglandin E2 (154,161). These osteoclasts activators presumably act on the osteoclasts and their precursors promoting osteoclast formation and osteoclast bone resorption. In addition, some of these cytokines can inhibit osteoblast differentiation and mineralization. The dual effects of inflammation lead to a substantial decrease in bone density subsequently resulting in osteoporosis. For a detailed review of how IBD affects bone health, please see elsewhere in this book (chapter X – Francisco Sylvester).

Enteritis

Pathogenic enteric infections may lead to drastic nutrient loss due to sustained diarrhea. These nutrient losses have been associated with a decrease in linear growth observed in children, where such infections are common (165). However, intestinal pathogens can also potentially affect bone growth through pathways other than reduced nutrient absorption. The immune response initiated by an intestinal pathogen may affect the process of long bone growth in children (165).

To study the response of the intestinal immune system to enteric pathogens, Noel et al, treated primary human macrophage-enteroid co-culture with enterotoxigenic and enteropathogenic *Escherichia coli* strains (ETEC and EPEC) (166). ETEC is the most

common pathogen that causes traveler's diarrhea while EPEC is associated with cases of fatal diarrhea in children (167,168). EPEC infection resulted in increased macrophage adherence to and projection across a filter into the enteroid monolayer; simulating an immune response where macrophages extend between enterocytes to interact with the pathogen. Macrophages present in ETEC infection also interacted with the bacteria through the filter and even reduced viable ETEC number, suggesting successful phagocytic action. In the absence of macrophages, overnight infection resulted in a loss of barrier function. However, their presence appeared to lessen this effect, further elucidating their protective role in such bacterial infections. While changes in intestinal cytokine expression levels of IFN γ , IL-8, and IL-6 have previously been implicated in affecting bone health (169), expression of these cytokines did not change in ETEC infection (166). This is further supported by the finding that ETEC infection is not associated with impaired linear growth (170). In contrast, enteroaggregative *E. coli* (EAEC) has been demonstrated to impair growth in infected children (171). Infected children showed a significant decrease in height when compared to the population. Interestingly, the decline in growth occurred regardless of diarrhea, discounting any effect of major nutrient loss. To examine a possible inflammatory mechanism, IL-8 levels were measured from stool samples and from EAEC-treated Caco 2 cells. IL-8 was elevated in children with persistent diarrhea and was shown to be increased upon exposure to two EAEC strains in *in vitro* studies (171). Considering previous studies on the role of IL-8 and bone (169), it is possible that an increase in this pro-inflammatory cytokine induced by EAEC infection results in reduced bone growth.

Furthermore, serum TNF α and IL-10 are elevated during enteritis from several bacterial species (172). In rotavirus and norovirus infections, IL-6 and IL-8 have been shown to increase according to the severity of disease (173). Although these studies do not reveal a bone phenotype in relation to the infection, these cytokines have been related to bone formation in other studies. For example, the combination of TNF α and IL-6 has been shown to induce bone resorption via differentiation of osteoclast-like cells (174). The role of TNF α in promoting osteoclast formation is well-established (95). In a model of childhood inflammatory bowel disease, a decrease in the proliferative zone of the tibia was observed and correlated with an increase in serum IL-6 concentration (175). It is possible that the reduction in bone growth seen in affected children is due to the activity of these cytokines. While pathogenic intestinal infection invokes immune responses such as increased macrophage projection and release of inflammatory cytokines, the associated decline in bone growth observed in some pathogenic infections in children is not clearly understood. Studies that measure bone parameters in addition to measuring inflammatory markers and other immune system responses are needed to accurately detail any effect of infection, if any, on bone health.

Dysbiosis and gut-bone immune link

Dysbiosis is characterized as “a compositional and functional alteration in the microbiota that is driven by a set of environmental and host-related factors that perturb the microbial ecosystem to an extent that exceeds its resistance and resilience capabilities” (176). This imbalance of “good” versus “bad” microbiota can be caused by various environmental factors: genetics of the host, diet, obesity, disease, or medical intervention (177). The use of

antibiotics for example, disrupts the composition of the gut microorganisms by reducing diversity and deleting or expanding bacterial communities (178). Specifically, streptomycin, one of the most commonly used antibiotics, reduced *Firmicutes* and depleted *Lactobacillus* spp, group D *Streptococcus* spp, *Enterococcus* spp but expanded *Enterobacteriaceae* (179); this demonstrates that the use of antibiotics can lead to dysbiosis. In addition to drug intervention, studies have shown that dysbiosis is observed in patients with a wide variety of diseases including: inflammatory bowel disease (Crohn's disease and ulcerative colitis) (180), asthma (181) and type II diabetes (182). Diet also plays a role in altering the composition of the gut-microbes. According to Zhang *et al.* (183), mice fed with a high-fat diet had a reduction in *Bifidobacteria* spp., while a high-fat and high-sugar diet increased the amount of *Clostridium innocuum*, *Catenibacterium mitsuokai* and *Enterococcus* spp. in the gut (184). In addition, obesity can also change the composition of gut microbiota. Obese humans tend to have a decreased abundance of *Bacteroidetes* compare to healthy humans (185). Weight loss in obese humans can restore the *Bacteroidetes* population (186) indicating that obesity has a negative impact on the composition of the human gut-microbiota, which is reversible. Taken together, these findings indicate that a variety of environmental factors can cause dysbiosis which can have serious consequences through altering the intestinal immune system and promoting inflammation (187). Furthermore, these changes can have far reaching effects as seen in bone health and the development of osteoporosis (188).

Dysbiosis also has negative influences on development and homeostasis of host tissue, including the bone (189). Specifically, dysbiosis can lead to the promotion of osteoclast differentiation and resorptive activity through the activation of pro-inflammatory T helper 17 (T_H17) cells, resulting in reduced bone mass (190). Moreover, Yan *et al.* (191) found that colonization of conventional specific pathogen-free gut microbiota in germ-free mice increased bone formation and resorption, suggesting that the gut microbiota can have a critical effect on bone remodeling. This link between dysbiosis and bone health can have important implications in infant and adult humans.

Pediatric disease and gut-bone immune link

The importance of the gastrointestinal tract and the associated microbiome in the maturation and development of the immune system has been demonstrated in studies utilizing germ-free (GF) mice. In the absence of the microbiome, germ-free mice have a) significantly fewer Peyer's patches that are hypoplastic and contain few germinal centers, b) smaller mesenteric lymph nodes and c) reduced lamina propria CD4⁺ T cells (192–194). Moreover, GF mice have less than one tenth of the number of IgA producing B cells compared to a conventional animal (193). Interestingly, exposure of the GF animals to a conventional microbiome results in the development of a 'normal' immune system, further supporting the key role of the intestinal bacteria in immune maturation (192,193). The gut bacteria have been demonstrated to influence the development of T_H17 cells, T_{regs} cells and memory T cells (195,196). Studies have shown that segmented filamentous bacteria are required for T_H17 development (197), and that members of the *Clostridium* spp induce colonic T_{reg} differentiation (198).

Emerging evidence supports that early life exposure to microbes may have an effect on later-life susceptibility to disease (199). Dysbiosis of the microbiome in young children can lead to the development of pediatric Crohn's disease (CD) and ulcerative colitis (UC) (200,201). Patients who develop these conditions as children or young adults are at an increased risk of bone fracture. Impairment of linear growth and failure to gain weight is common in children with CD and UC. As a consequence of this these patients have a reduced peak bone mass with significantly lower bone mineral density and bone mineral content, significantly increasing the risk of developing osteoporosis in later life (202).

Aging and the intestinal / bone immune systems

In the elderly both the bone and gut immune systems undergo age-related alterations. This age-related change in the immune system is known as immunosenescence, which contributes to the increased susceptibility of the elderly to infectious disease, cancer and autoimmunity (203). Immunosenescence affects various cell types located in the bone marrow and thymus, lymphocytes located in secondary lymphoid organs and the peripheral blood as well as aspects of the innate immune system (203–205). In bone this change can lead to bone loss resulting in deteriorated structure and function and eventually osteoporosis (206). While in the intestine this can result in a low grade inflammatory status, potentially developing into a more severe pathological condition such as IBD. This can subsequently have adverse effects on bone health (207).

During aging, the hematopoietic compartment of the bone marrow decreases in size and is replaced by fatty deposits (208). Unexpectedly the number of hematopoietic stem cells (HSCs) in the bone marrow increases with age however, these cells exhibit several functional defects including diminished regenerative potential (209). Aging HSCs also exhibit skewed differentiation bias with decreased output of lymphoid and erythrocyte lineage cells. In contrast, output of myeloid cells has been reported to decrease, remain the same or increase (204,209,210). However, aging has been shown to impair the ability of bone marrow macrophages to secrete cytokines such as TNF α , IL-1 β and IL-6 (210). The number of T cell and B cell progenitors present in the bone marrow and thymus is also significantly reduced with age. The progenitors are also of an inferior quality when compared to their young counterparts as they exhibit reduced proliferative ability and increased apoptosis (211–213). Aging has also been shown to suppress expression of IL-7 by bone marrow stromal cells, a cytokine that is essential for the survival of developing lymphocytes (205,214). What direct effects these changes in bone marrow immune function have on bone health is not well understood.

In addition to changes in the bone immune system, the gastrointestinal lymphoid tissue (GALT) also undergoes significant age-induced modifications which is likely a major factor in the increase in the incidence and severity of infections in the elderly (215). Studies have observed that aging is linked to decreased Peyer's patch size; this is potentially due to impaired movement of immune cells in and out of the Peyer's patches (216). Studies by Ogino et al (217) and Schmucker et al (218) report reduced immune cell migration with age. Further evidence for age-induced changes in the gastrointestinal immune systems is seen in the reduced number of intraepithelial regulatory-type T cell subsets and reduced expression

of TGF β and IL-10 (219). Whether these effects of aging on the gut immune system directly affect bone marrow immune function and if this impacts bone health negatively is not well known.

The Effect of Antibiotics on Gut-Bone Immune Signaling

The use of antibiotics in the past 80 years has saved millions of human lives from bacterial infection. However, oral antibiotic treatment kills both pathogenic and commensal microbes. Commensal microbes have been shown to perform an array of important functions in the human body including regulation of bone health (220,221). Thus it is conceivable that oral antibiotics can impair gut immune function that can negatively influence bone health. While there is evidence for this, the mechanisms of how antibiotic treatment alters the interaction between the microbiome and the immune system is not well understood. There have been relatively few studies that have investigated the effect of antibiotic depletion of the gut microbiome on bone health. In a study by Cho et al (222), weaned female C57BL/6 mice were treated with antibiotics (penicillin, vancomycin, chlortetracycline) for 3 weeks and displayed an increase in total body bone mineral density (BMD) compared to the untreated mice. Interestingly, after 7 weeks of treatment, BMD was comparable between the treated and untreated groups (222). Cox et al (223) treated C57BL/6 male and female mice with low dose penicillin for 20 weeks (either before birth or from weaning) and found a small but significant increase in bone mineral content (BMC) and BMD in female mice. In contrast, males, given antibiotics before birth, exhibited a significant decrease in BMC (223). These effects were thought to be linked to the microbiome affecting the differentiation of stem cells into osteoblasts, adipocytes or myocytes. Taken together, these studies emphasize the complexity of bone responses to antibiotic treatment and highlight the importance of variables such as length of treatment, mouse strain/sex and age, and potentially mouse microbiome.

Conclusion

The intestine is residence to the largest number of immune cells in the body. These immune cells are intricately associated with the intestinal microbiota and subsequent changes in the microbiota can have both local and systemic beneficial or adverse effects. Recent studies implicate a gut–bone axis where changes to the intestine can affect distal bone health. Indeed, dysbiosis of the intestinal microbiota, either through disease, aging or antibiotics, can alter the intestinal immune system leading to increased inflammation and subsequently resulting in bone loss and the development of osteoporosis.

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Fig 1. Regions of the Intestine

The intestine is composed of two main segments: the small intestine and the large intestine. The small intestine is further divided into the duodenum, jejunum and ileum while the large intestine consists of the cecum, colon and anus. These regions are specialized with regard to the digestion and absorption of nutrients required to maintain homeostasis.

Fig 2. Layers of the Intestinal Tract

A cross section of the intestine displaying the different layers. The serosa, the outermost layer, covers the intestine. The muscular layer (muscularis externa) consists of two types of muscle, the inner circular muscle and the outer longitudinal muscle; coordinated contraction of these muscles, known as peristalsis, moves chyme / feces through the intestinal tract. The submucosa consists of dense and irregular connective tissue containing blood vessels, lymphatics and nerves that branch into the mucosa. The mucosa is the inner most layer of the intestine and consists of the epithelium (in contact with the lumen), the lamina propria and the muscularis mucosae.

Fig 3. Simplified Schematic Representation of the Intestinal Layers and Epithelial Barrier of the Small (left) and Large (right) Intestine

The small intestine consists of projections in to the lumen known as villi. These vastly increase the absorptive surface area. In contrast, the colon is relatively smooth and contains crypts. The epithelial barrier is made up of numerous cell types. Enterocytes, found in the small intestine, are the absorptive cell and have microvilli on their apical surface further increasing the surface area involved in digestion. Interspersed between the enterocytes are goblet cells and intraepithelial lymphocytes (IELs). Goblet cells produce mucus that forms a protective layer over the epithelial barrier. In the small intestine this mucus layer is loosely adherent while in the large intestine the mucus layer is split into two sections; a loose outer layer and an adherent inner layer. IELs have numerous functions including but not limited to; forming a frontline of defense against pathogens in the lumen, suppressing excessive inflammation and ensuring the integrity of the epithelium. Within the intestinal crypt Paneth cells produce antimicrobial peptides, defensins and lysozymes. These Paneth cells are interspersed between stem cells which renew the intestinal epithelium. Adapted from Mowat et al. (1).

Fig 4. Simplified Schematic Diagram of the Peyer's Patch

Peyer's patches are lymphoid structures visible on the anti-mesenteric side of the small intestine and are composed of B cell germinal centers surrounded by T cells. Peyer's patches are characterized by the presence of the follicle-associated epithelium which contains M cells. M cells transport the antigens present in the lumen into the Peyer's patch where they are up taken by DCs. DCs present these antigens to the lymphocytes which initiate the adaptive immune response. Adapted from Mowat et al. (23).

Fig 5. Immune Cells of the Lamina Propria

The lamina propria is comprised of loosely packed connective tissue that forms a supportive structure for the villi and contains the mucosa blood supply, lymph drainage and nervous system. The lamina propria cell population is variable containing cells of the innate immune system: DCs, macrophages, eosinophils and mast cells, and adaptive immune system: T lymphocytes ($CD4^+$ T_H1 , T_H2 , T_H17 , T_{reg} and T_R1 ; $CD8^+$) and B lymphocytes (B cells and IgA and IgM producing plasma cells). Adapted from Mowat et al. (1).

Fig 6. Osteoblast and Osteoclast Cross Talk

Osteoblasts regulate osteoclast differentiation and activity through the expression of three critical cytokines: macrophage colony stimulating factor (M-CSF) receptor activator of nuclear-factor kappa B ligand (RANKL) and osteoprotegerin (OPG) a soluble decoy receptor for RANKL. Conversely, osteoclasts can modulate osteoblast differentiation through expression of the osteogenic factors Wnt10b and bone morphogenic protein (BMP)-6.

Fig 7. Lymphocyte Modulation of Bone Remodeling

Bone marrow lymphocytes can modulate the differentiation and function of osteoclasts and osteoblasts through the expression of pro- and anti-inflammatory cytokines as well as RANKL and OPG.

Fig 8. Role of Myeloid Cells in Osteoclast Differentiation

Under inflammatory conditions neutrophils upregulate expression of RANKL which can stimulate osteoclast differentiation. Furthermore, following stimulation with RANKL and MCSF, immature dendritic cells can transdifferentiate into functional osteoclasts.