

PATHOPHYSIOLOGY OF OSTEOPOROSIS – A REVIEW

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Osteoporosis is a disease characterized by low bone mass, micro-architectural deterioration of bone tissue leading to enhanced bone fragility, and a consequent increase in fracture risk. It is a major cause of morbidity and mortality and medical expense worldwide. In India, it has been found that 29.9% of women and 24.3% of men aged between 20 and 79 years have low bone mass. There are different mechanism for the osteoporosis like, The Role of RANK/RANKL/OPG and T Cells in Bone Remodeling, Chronic Immune Activation and the Uncoupling of Remodeling, Oral Tolerance and Bone Health, T-Helper 1 (Th1), Toll-like Receptors, Involution of Thymus Gland and the Beginning of Bone Loss.

Keywords: Osteoporosis, RANK/RANKL, oral tolerance.

INTRODUCTION

Osteoporosis is a disease characterized by low bone mass, micro-architectural deterioration of bone tissue leading to enhanced bone fragility, and a consequent increase in fracture risk. It is a major cause of morbidity and mortality and medical expense worldwide. In India, it has been found that 29.9% of women and 24.3% of men aged between 20 and 79 years have low bone mass. Furthermore, about 50% women and 36% of men over 50 years of age are noted to have low bone mass. Osteoporosis affects an estimated 75 million people in the United States, Europe, and Japan combined, including one in three postmenopausal women and majority of the elderly. Osteoporosis causes more than 1,300,000 fractures annually in the United States alone. The disease will be a greater problem in the future, because the world population is aging and the incidence of osteoporotic fractures is increasing in much geographic area¹.

The primary osteoporosis includes two entities: one related to menopause, estrogen loss and the other to aging. The primary osteoporosis represents two fundamentally different conditions. Type I characterized by

loss of trabecular bone owing to estrogen deficiency at menopause and type II osteoporosis characterized by loss of cortical and trabecular bone in men and women owing to long-term remodeling, inefficiency, dietary inadequacy, and activation of the parathyroid axis with age².

PATHOPHYSIOLOGY

Bone remodeling comprises the coordinated processes of bone resorption and formation where old bone is replaced by new bone^{3,4}. Osteoclasts differentiated cells specialized to resorb bone (Teitelbaum, 2000), originate from the macrophage/monocyte lineage and differentiate to multinucleated cells, with their proliferation, differentiation and fusion being under the control of many hormones and cytokines. On the other hand, osteoblasts that form bone derive from mesenchymal stem cells (MSCs) that undergo differentiation through multiple steps, with final terminal differentiation into osteocytes. Due to the large number of bone cell types, stages of differentiation, and the numerous growth factors and cell-cell interactions involved, understanding of bone remodeling and the

tight coupling between osteoblasts and osteoclasts is still fragmentary. The following different mechanisms are responsible for the osteoporosis.

(1) The Role of RANK/RANKL/OPG and T Cells in Bone Remodeling

During normal bone remodeling, marrow stromal cells and osteoblasts produce RANKL, which binds to the transmembrane receptor RANK on osteoclast precursors and induces differentiation and activation⁵. This occurs through the transcription factor, nuclear-factor kappa B (NFκB), which is responsible not only for activating osteoclastogenesis but also the body's inflammatory response. Both osteoclast

differentiation and the inflammatory process occur via regulation of interleukin-6 (IL-6). Both OPG and RANKL are mainly produced by bone marrow stromal cells and osteoblasts. RANK, present at the surface of the osteoclastic lineage, is the third protagonist and represents the receptor for RANKL. In this triad, OPG acts as a receptor antagonist by binding both the soluble and the cell-bound forms of RANKL, prevents their interaction with RANK and inhibits the osteoclast differentiation/activation. The deregulation of this molecular triad is in part responsible of the osteolysis associated to malignant tumours, and their development in bone site (Figure 1).

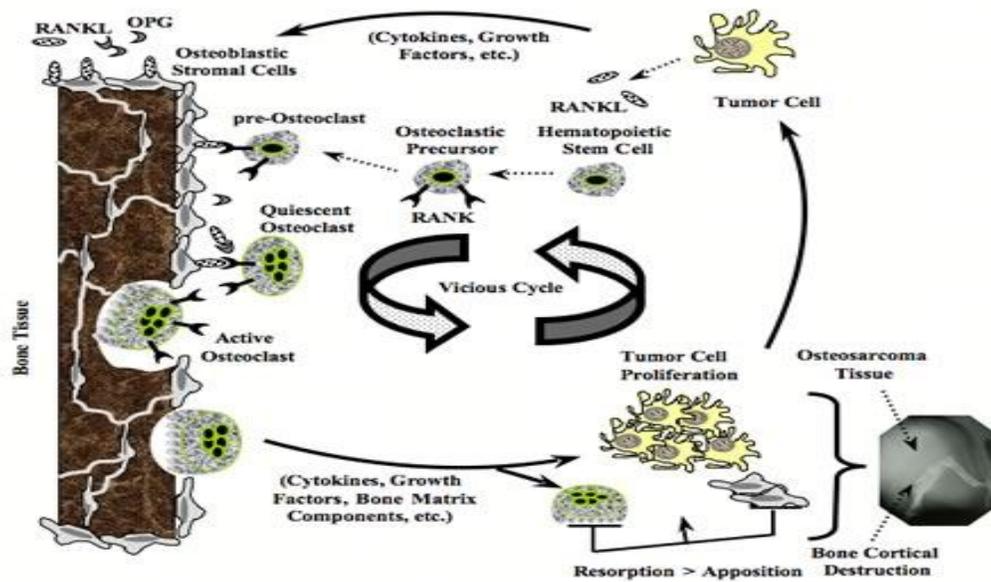


Fig.1: Involvement of the molecular triad OPG/RANKL/RANK in the bone tumor associated osteolysis⁶

Recent experiments were performed to measure the effect of RANKL and OPG on metalloproteinase (MMP), cystein protease (cathepsin K) and TRAP activities in bone cells [total bone cells (Figure 2), purified osteoclasts from peripheral blood or bone marrow and murine osteoclast precursor RAW 264.7 cells. These data associated with the results obtained by western blot (signal transduction induced by OPG) demonstrated that OPG is not a simple decoy receptor for RANKL, but

rather represents a new effector of osteoclast biology. Surface plasmon resonance (BIA core system) experiments revealed the capacity of OPG, RANKL and RANK to form a ternary complex. Moreover, OPG interaction with glycosaminoglycans (heparin, heparan sulfate, dermatan sulfate, and chondroitin sulfate) prevents its binding to RANKL (Figure 3), suggesting an essential function of proteoglycans in the activities of the triad OPG/RANK/RANKL (Figure 4).

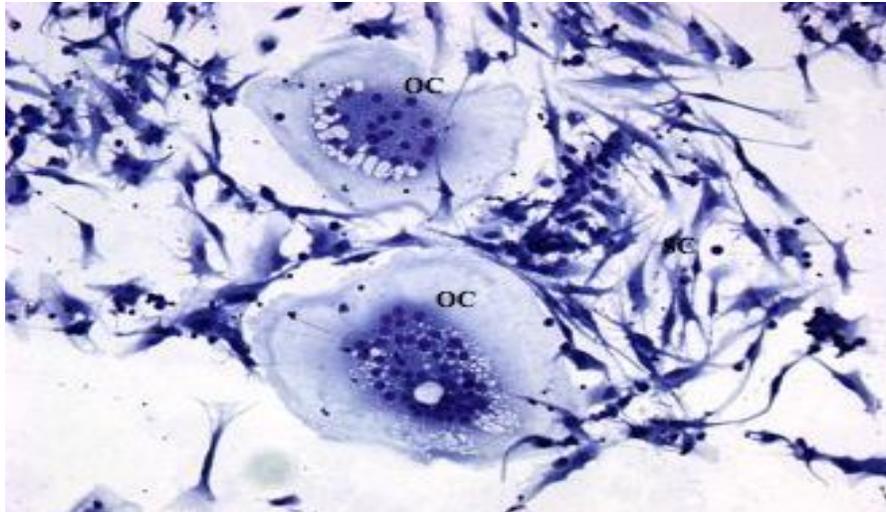


Fig.2: Primary culture of total rabbit bone cell (OC: osteoclast; SC: Stromal cells)

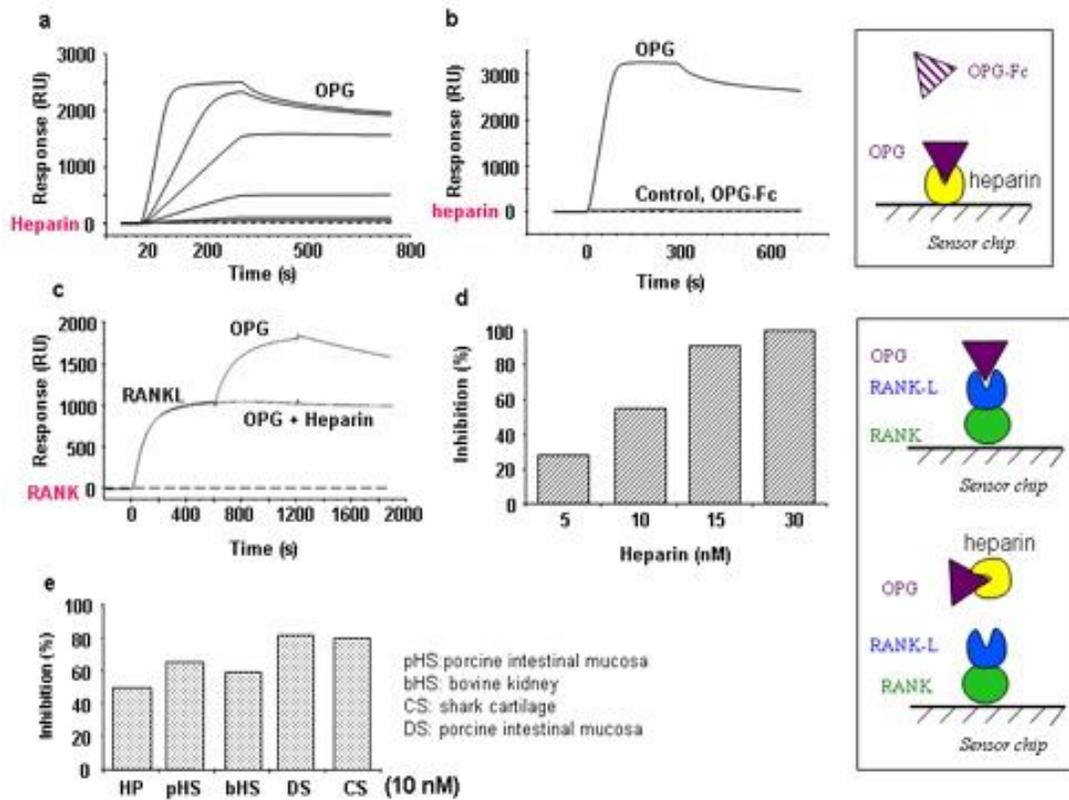


Fig.3: Characterization of osteoprotegerin binding to glycosaminoglycans by surface plasmon resonance: role in the interactions with Receptor Activator of Nuclear Factor kB Ligand (RANKL) and RANKL⁷

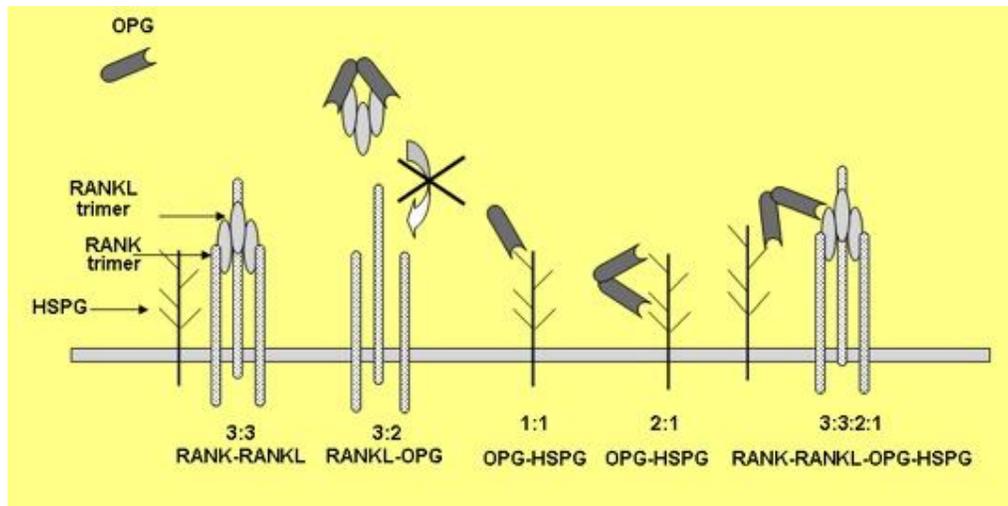


Fig.4: Characterization of osteoprotegerin binding to glycosaminoglycans by surface plasmon resonance: role in the interactions with Receptor Activator of Nuclear Factor κ B Ligand (RANKL) ⁷

(2) Chronic Immune Activation and the Uncoupling of Remodeling

RANKL is also produced by activated T cells. With reduced estrogen levels and/or chronic or recurrent immune activation from either systemic or gastrointestinal origin, there may be a reduction in the body's natural ability to limit the production of RANKL⁸. This results in increased osteoclast activation through a "switch-like" diversion of osteoprogenitor-cell differentiation away from monocyte-macrophage-cell development and toward osteoclastogenesis. Osteoclastic activity, induced by proinflammatory cytokines and activated

T cell-induced RANKL, is thought to be modulated by the action of interferon gamma on tumor necrosis factor receptor-associated factor 6 (TRAF-6)⁹. TRAF-6 is a RANK adaptor protein that mediates NF κ B activation^{10,11}. This modulating capacity of IFN γ over RANKL is influenced by both vitamin D and estrogen. Aging leads not only to a reduction in sex-hormone production, but also to an increase in the general level of proinflammatory cytokines and diminution of immune system function. In vivo, free radicals have been shown to increase bone resorption¹², and oxidative stress reduces BMD in humans¹³. These environmental and/or age-related catabolic stressors contribute to normal bone loss. But when there is chronic, elevated antigenic load or excessive oxidative

stress, which increases proinflammatory cytokine-induced RANKL, the activation of this "switch" in osteoprogenitor-cell differentiation may, independent of age, adversely affect the balance of bone remodeling. It is in this abnormal state that chronic immune activation may alter IFN-modulating capacity. When estrogen is deficient, causing RANKL levels to increase, the body's natural ability to limit the transcription factors TRAF-6 and NF κ B may be reduced and IFN- γ may exert a pro-osteoclastogenic effect¹⁴.

This uncoupling of the remodeling process results in bone loss. In studies using mice, chronic antigenic load with T-cell activation and production of reactive oxygen species (ROS) must be present for low estrogen levels to cause bone loss¹⁵. It appears that reducing antigenic load and oxidative stress may be equally as important as estrogen in maintaining bone health.

(3) Oral Tolerance and Bone Health

Oral tolerance, the muted immunological response to harmless gut antigens, depends on the presence of commensal microorganisms and an intact healthy gut wall. Epithelial cell integrity is maintained by the presence of beneficial organisms such as Lactobacillus and Bifidobacteria that do not elicit an inflammatory response. When normal gastrointestinal flora are maintained,

immunological self-tolerance through the activation of T-regulatory cells (Tregs) favors a non-inflammatory T-helper 2 (Th2) dominant response to gut microbes¹⁶. Pathogenic bacterial or fungal overgrowth causes inflammation and increased gut permeability that reduces oral tolerance. Focus on the traditional osteo-endocrine explanation for bone homeostasis fails to acknowledge the important role of the immune system in remodeling and the possible role of oral tolerance in maintaining bone health. It is now understood that a high systemic antigen load of bacterial or viral origin and/or a loss of oral tolerance due to pathogenic microbial overgrowth (long suspected as major contributing factors in other chronic degenerative diseases) may also contribute to the pathogenesis of bone loss.

Estrogen normally helps preserve bone by enhancing macrophage production of transforming growth factor β (TGF- β) and limiting CD4+ T-cell activation. Reduced levels of estrogen result in an increase in antigen-presenting cells and a reduction in TGF and Tregs. This leads to T-cell activation and production of proinflammatory cytokines and RANKL, which stimulates osteoclastogenesis. By improving gut health and oral tolerance, antigen presentation to cells is reduced, TGF production is maintained, Tregs are enhanced, and RANKL-induced osteoclastogenesis is limited, even with reduced levels of estrogen

(4) T-Helper 1 (Th1)

Though infection, inflammation, and autoimmune disorders are known to be associated with bone loss, only recently has it been recognized that T lymphocytes and their products are the key regulators for bone remodeling^{17,18}. Major advances and discoveries in this field have led to revelations about the molecular mechanisms, various cytokines, and signaling transducers that participate in the regulatory interactions between T lymphocytes and bone cells^{19,20}. Moreover, besides the arsenal of mutual signaling molecules, T lymphocytes and bone cells also share a common site of origin, namely bone marrow (BM). They influence each other not only after maturation and activation, but also at the very beginning of their existence. For example, T cells are capable of affecting osteoclastogenesis by secreting various cytokines such as interleukin

(IL)-1, IL-6, interferon (IFN)- γ or IL-4^{21,22}. Herein, we will review the direct and indirect mechanisms involved in osteoporosis and the evidence to support the hypothesis that one of the critical mechanisms involved in osteoporosis is activated T cells induce the production of osteoclastogenic factors.

Double-edged Sword Effects of T Cells upon Osteoclastogenesis

The BM hosts fully functional and mature T cells that exhibit several distinctive features. In the BM, mature T cells represent about 3%-8% of total nucleated cells²³. BM not only primes naive T cells and recruits effector T cells, but also serves as a site for the preferential proliferation of CD4+ and CD8+ T cells²⁴⁻²⁸. BM T cells contribute to the homeostasis of the immune system and to the bone cells present in BM environment. Depending on whether studies are performed in vitro or in vivo, T cells exert varying effects on osteoclastogenesis.

Promoting Effects of T Cells on Osteoclastogenesis

Activated T cells may undermine bone homeostasis and stimulate bone destruction under pathological conditions such as estrogen deficiency²⁹⁻³². They have exerted their effect via membrane-bound and secreted RANK ligand or RANKL, an essential stimulating signal for osteoclastogenesis that is involved in activating mature OCs. Transfer of *ctla4*^{-/-}-bone marrow cells in *rag1*^{-/-} mice led to a significant decrease in BMD. Consistent results were achieved by direct transfer of purified *ctla4*^{-/-} T cells in *rag1*^{-/-} and *opg1*^{-/-}-mice²⁹. In another elegant study, Cenci et al reported that increased production of TNF- α by T cells in bone marrow mediated the increased bone resorption and bone loss in ovariectomized mice. Ovariectomy-induced bone loss is prevented by administering either estrogen, TNF- α -binding protein, or an anti-TNF- α antibody. TNF- α augments M-CSF- and RANKL-induced OC formation. But in T-cell deficient mice, ovariectomy failed to induce bone loss, stimulate bone resorption, or increase M-CSF- and RANKL-dependent osteoclastogenesis³⁰. By utilizing DO11.10 mice, a strain in which all T cells recognize a single peptide epitope of chicken albumin, they also investigated the effect of estrogen deficiency upon the birth and death of cytokine-producing T cells. Their studies

revealed that estrogen deficiency induced osteoporosis by increasing T cell activation-induced proliferation, and suppressing the apoptosis of active T cells, while the blockade of antigen presenting cell (APC)-induced T cell activation prevented the resulting T cell expansion and bone loss³². Consistent with the results of animal studies, a human study of post-menopausal bone loss demonstrated women with post-menopausal osteoporosis have higher T cell activity than healthy post-menopausal subjects³³. Among T cells, a T helper cell 17 (Th17) cell subset is important in inducing bone loss, which contrasts with the more established T lymphocyte cytokine-expressing subsets- Th1 and Th2. Recent data indicate that the IL-17-producing Th17 cell subset stimulates osteoclastogenesis through osteoclastogenesis-supporting cells, which is the only osteoclastogenic Th cell subset characterized so far³⁴. IL-17, a cytokine secreted by the Th17 cell, is well known to induce local inflammation in autoimmune diseases through inflammatory cytokine production. Moreover, IL-17 induces RANKL expression that is crucial for osteoclastogenesis and bone resorption. In addition, it can synergize with these cytokines (IL-1, TNF- α , RANKL), but has direct activity as well. Th17 cells express higher levels of RANKL than Th1 and Th2 cells³⁴. Therefore, the infiltration of Th17 cells into the inflammatory lesion links the abnormal T cell response to bone damage. Therefore, given that activated T cells have promoted osteoclastogenesis in vivo, this subset is an auspicious therapeutic target for bone loss.

Interdicting Effects of T Cells on Osteoclastogenesis

On the other hand, T cells are capable of mediating anti-osteoclastogenic signals in bone turnover. Hints that this modulation may occur come from in vitro studies demonstrating that osteoclastogenesis was inhibited by CD8+ T cells^{35,36}. Moreover, after activation, mouse CD8+ T cells showed delayed kinetics of RANKL expression, as compared with corresponding CD4+ T cell³⁷. Depletion of CD4+ and CD8+ T cells in mice enhanced vitamin D3-stimulated OC formation via a mechanism involving decreased osteoprotegerin (OPG) production³⁸. The protective role of T cells on bone homeostasis was also clearly documented by other in vivo studies that

showed that both B cell- and T cell-deficient mice had decreased BMD³⁹. Furthermore, researchers found OPG production in the BM by B cells stimulated by T cells through CD40L/CD40 interactions prevented osteoporosis. IFN- γ produced by T cells potently suppressed RANKL signaling by accelerating the degradation of TRAF6 by activating the ubiquitin/proteasome system. Additionally, anti-CD3 and anti-CD28-Ab activated T cells and inhibited osteoclastogenesis, while activation of T cells by staphylococcal enterotoxin A, phytohemagglutinin and concanavalin A had inconsistent effects.

T Helper Cells and Osteoclastogenesis

Depending on the manner in which they are activated, T cells can mediate both osteoclastogenesis and anti-osteoclastogenesis⁴⁰. The net effect of T cells on osteoclastogenesis depends on the balance between positive and negative factors expressed by the T cells. While the CD4+ Th cell subsets Th1 and Th2 produce IFN- γ and IL-4, respectively, both of which are anti-osteoclastogenic. It is unclear how activated CD4+ Th cells in arthritis enhance osteoclastogenesis in the presence of these cytokines. Therefore, a need exists to define the very rare but pathologically important Th cell subset responsible for abnormal bone resorption, such as osteoclastogenic Th cells (Thoc cells). As indicated by Tomoki et al, the characteristics of Thoc cells should be the following: First, Thoc cells do not produce a large amount of IFN- γ . Second, they trigger local inflammation and the production of inflammatory cytokines, including TNF- α , to induce RANKL expression on synovial fibroblasts. Third, Thoc cells express RANKL and might directly participate in accelerated osteoclastogenesis⁴¹. If these Th cells have such characteristics as those above, they may be more disposed to osteoclastogenesis over anti-osteoclastogenesis.

(5) Toll-like Receptors

Toll-like receptors (TLRs) are proteins on the outside of certain cells that identify and help destroy disease-causing organisms (called pathogens) that have entered the body. They are present in areas of the body that are frequently exposed to outside substances such as the skin, nose, eyes, mouth, or gastrointestinal tract. TLRs are part of a larger

group of proteins called pattern-recognition receptors (PRRs). These proteins are key components in the body's innate immune system. This branch of the immune system is the body's first line of defense against foreign substances trying to enter the body and cause illnesses. All humans are born with innate immunity. This type of immune response is both immediate and nonspecific. In other words, immune cells involved in innate immunity are not specific to just one type of foreign substance. Instead, the immune cells can recognize and destroy a wide range of pathogens. PRRs, including toll-like receptors, are constantly surveying the body for pathogens, such as bacteria and viruses. They are able to recognize disease-causing organisms (such as bacteria or viruses) by their pathogen-associated molecular patterns (PAMPs). PAMPs are cellular patterns that are shared by a large group of organisms. Since these patterns are different than human cells, they will not mistakenly attack body cells. TLRs are named after a closely related receptor in the fruit fly, called a toll receptor. Both the human TLRs and the fruit fly toll receptors stimulate the immune response, which leads to the destruction of the invading pathogen.

TLRs are predominantly found on the outside of antigen presenting cells (APC), including neutrophils, macrophages, and dendritic cells. When the TLR identifies a foreign invader, the APC engulfs it. The APCs then break down the pathogen so that other immune cells (such as T-cells and CD4 cells) can identify and destroy the harmful invader. In addition, TLRs have been found in a wide range of body tissues, including the adrenal glands, liver, testis, spinal cord, lungs, thymus gland, and trachea. This suggests that most cells in the body express TLRs to help fight against diseases and infections.

The production of gut-related proinflammatory cytokines is reduced by the maintenance of a healthy gut flora. Toll-like receptors are transmembrane receptors found on macrophages, dendritic cells, and some epithelial cells, and play an integral role in maintaining oral tolerance. These receptors recognize the molecular patterns of bacteria and elicit an inflammatory, destructive response to pathogenic microbes and a tolerogenic response to commensal bacteria. An example of how a disease-related genetic polymorphism can be influenced through the

reduction of metabolic stressors can be seen in the case of toll-like and IL-1 receptors. Because the cytoplasmic portion of the toll-like receptor is similar to that of the IL-1 receptor, an individual suffering from chronic dysbiosis and also carrying the polymorphism for the IL-1 receptor antagonist gene could, in theory, be susceptible to an increased diversion or "switch" of cells from the monocyte-macrophage cell line to form osteoclasts. A reduction of antigen load and oxidative stress, no matter the cause (e.g., insulin/glucose imbalance, toxicity, or gut pathogenic microflora), could reduce proinflammatory cytokine-induced chronic inflammation and T-cell activation

(6) Involution of Thymus Gland and the Beginning of Bone Loss

Reduced oral tolerance may be a factor in the apparent coincidence between thymus gland involution (and subsequent reduction of naive T-cells) and the onset of bone loss that begins in humans in their mid-30s. Although BMD does not usually decrease significantly until menopause, accelerated bone loss can commence at an earlier age for some individuals. Reduced numbers of naive T cells from chronic systemic inflammation or antigen overload from the gut leads to oligoclonal T-cell expansion and increased T-cell senescence⁴². Senescence reduces a T cell's ability to produce IFN- γ and is a sign of immune aging.

The primordial thymus developed as a bud on the immature digestive tract, providing embryological evidence of the uniquely co-dependent and interrelated functions of the thymus gland and gastrointestinal tract⁴³. As an infant grows, the function of the thymus is to relieve the gut of its primordial function of lymphopoiesis. 52 With involution of the thymus, the adult gastrointestinal tract remains the source of at least 75 percent of the body's immune cells;⁴⁴ therefore; it is in the gut that an adult's immune health is maintained or lost. As an individual ages, antigen load often increases and oral tolerance decreases, leading to reduced levels of IL-2 (necessary for T-cell proliferation and differentiation into activated [effector] cells) and IFN- γ , and ultimately to a greater cache of RANKL-expressing (and thus osteoclast-activating) memory cells harbored in the bone marrow.

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