IMMUNOLOGICAL ASPECT ON BONE CELLS

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ABSTRACT

Immunological aspect on bone cells is closely connected with the existence of estrogen. There are so many immune factors depend on estrogen. Functional interdependence between immune and bone systems is reflected in a number of regulatory molecules acting on the cells of both systems and common precursors for bone and immune cells. Therefore, the disturbances of the immune system may affect bone metabolism, and vice versa. This review addresses the roles of two major immune cell populations, T and B lymphocytes, in the regulation of bone metabolism. Experimental models and human diseases demonstrated that T lymphocytes may produce many bone cell regulatory cytokines, including two essential stimulators of osteoclastogenesis: receptor for activation of nuclear factor kappa b (NF-κB) (RANK) ligand (RANKL) and macrophage colony-stimulating factor (M-CSF). This journal will reveal some of estrogen roles on bone immunology system that can affect bone metabolism.

***Keywords**: Immune system, bone cells, estrogen*

INTRODUCTION

Postmenopausal osteoporosis is a major cause of skeletal morbidity, leading to more than 1.3 million pathological fractures per year. Approximately 10 million postmenopausal women in the United States have osteoporosis [bone mineral density (BMD), >2.5 SD below the young normal mean], and another 17 million have osteopenia (BMD, >1 SD below the mean). Antiresorptive agents, including estrogen, are beneficial for many of these patients. These resorption inhibitors protect existing bone and cause modest (2–9%) increases in bone mass (Kostenuik et al, 2001).

The importance of estrogen deficiency in the rapid bone loss around the time of menopause has been recognized for many years. Studies of younger women with amenorrhea as a result of intense athletic activity**,** anorexia nervosa**,** and other causes have shown that estrogen deficiency at any age may also be associated with bone loss**,** although many of these conditions are more complicated than simple estrogen deficiency. Moreover**,** women with late onset of menarche appear to have significantly reduced peak bone mass. Among perimenopausal women**,** serum estrogens are lower among women with more rapid bone loss, and serum osteocalcin concentrations as a marker of rates of skeletal turnover**,** are higher in perimenopausal women with lower estrogen concentrations and more rapid bone loss (Slemenda et al, 1996).

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However**,** less is known regarding the important role of estrogen in two interconnected systems, immune system and bone. When estrogen deficiency happens, it can lead to several interactions between T and B-lymphocyte, as the part of immune system, with bone cells, which can produce osteoporosis. To address this issue, here the author will try to present several studies related to that subject and finally making her own deductive hypothetical conclusion in the end of this review. Hopefully, this review can be useful for further studies that will be made in the future related to better treatment for osteoporosis.

DISCUSSION

The load-bearing bones are like busy interstate highways, which become cracked and worn with use and will crumble unless continuously repaired by maintenance crews. Bones too have repair or remodeling crews -- the basic multicellular units (BMUs). Although bones may look inert, they need a lot of repair and renewal and are thus constantly seething with activity. Every 10 seconds a BMU crew is activated. At any time, about 35 million are at work digging holes and removing and replacing about 500 mg of calcium throughout the skeleton each day. This ongoing digging throughout the skeleton creates the so-called "remodeling space." However, the remodeling rate varies widely throughout the adult skeleton. For example, the remodeling rate in adult cortical bone can be as low as 2% per year in the distal radius and as high as 50% per year in ileal trabecular bone, which is immediately accessible to the nests of osteoclast and osteoblast precurors in the bone marrow stroma (Cenci et al, 2000; Whitfield, 2001).

BMUs are not as good at patching as road repair crews, which is why a woman's once strong koric (from the Greek word *kore*, meaning "young woman") bone weakens with advancing age. The availability of osteoblasts for BMUs declines with advancing age as the number of osteoblast progenitors in, and the osteogenic potential of, the bone marrow stroma decline. Osteoblasts working on the inner surface of the cortical bone and the trabeculae in the cancellous compartment do not completely refill the osteoclasts' excavations, but fortunately periosteal osteoblasts tend to overfill the holes in the periosteum. Therefore, with advancing age, cortical shells thin while the overall diameter of a long bone increases, which resists an increase in a bone's vulnerability to bending and breaking. But there is an exception -- the femoral neck. The cortex of the femoral neck thins, but its diameter does not increase because there are no periosteal BMUs. Consequently, the aging hip becomes especially vulnerable to bending and breaking and, consequently, must be one of the targets of any successful anabolic drug. The decline in bone mass in women accelerates when the estrogen level drops below a critical level at menopause. Because of an osteoclast population explosion, and because there are fewer osteoblasts to fill the holes, bone turnover increases as does the remodeling space (ie, the total volume of unfilled or partially filled osteoclast excavation sites) -- perhaps enough to make bones vulnerable to being broken by the muscle-imposed strain of once-normal body movements (Cenci et al, 2000; Grcevic et al, 2001; Whitfield, 2001).

Estrogen Effect on Bone Cells

A young woman's estrogen is responsible for many osteoclast-suppressing activities in her bones (Figure 1). Estrogen limits the size of her preosteoclast and osteoclast population by a transforming growth factor (TGF)-beta-mediated killing of these cells by apoptosis and by stimulating osteoblastic stromal cells to make the osteoclast-suppressor osteoprotegerin (OPG) (Figure 1). Estrogen also limits osteoclast production by restraining the production and secretion into the marrow stroma of interleukin (IL)-1beta, which otherwise would stimulate marrow monocytes and T-cells to make tumor necrosis factor (TNF)-alpha, a stimulator of osteoclast development (Figure 1). To ensure that IL-1beta does not stimulate osteoclast progenitors and T cells, estrogen induces them to express nonsignaling type 2 IL-1beta decoy receptors that prevent these cells from responding to IL-1beta and secreting TNF-alpha, which drives osteoclast differentiation and osteoblast apoptosis (Figure 1). Estrogen also restrains the production of the potent osteoclast generators macrophage colony-stimulating factor (M-CSF) and the IL-6 and IL-11 interleukins (Figure 1) (Grcevic et al, 2001; Jilka et al, 2001; Whitfield, 2001).

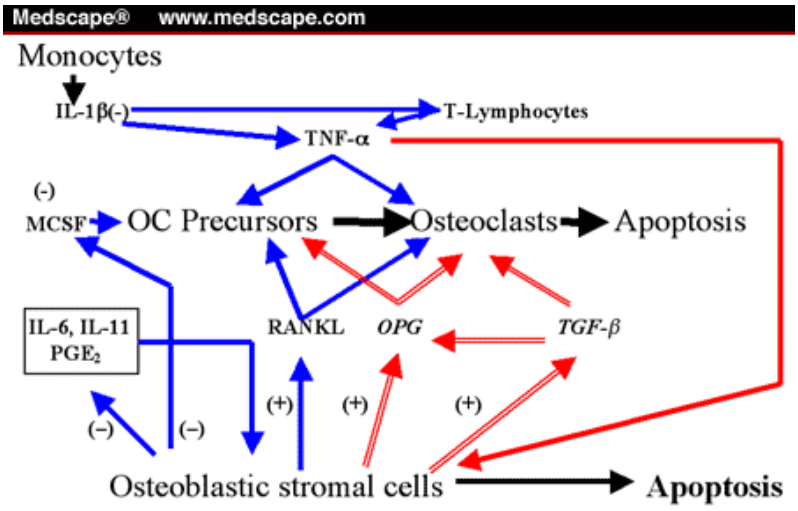

Figure 1. How estrogen restrains osteoclasts. **(-)** means that estrogen restrains the factor; **(+)** means that estrogen promotes the secretion of the factor. A blue arrow indicates that the factor stimulates its target cell; a red arrow indicates that the factor inhibits or kills its target. OC precursors = osteoclast precursors; MCSF = macrophage colony-stimulating factor (osteoclasts belong to the macrophage lineage) (Whitfield, 2001).

Osteoblastic stromal cells make both the anti-osteoclast OPG and the osteoclast-stimulating receptor activator of NF-kappaB ligand (RANKL). But in the presence of estrogen and TGF-beta (1 and 2), the osteoblastic stromal cells synthesize more OPG than RANKL (Figure 1). The secreted OPG is a soluble decoy RANK-like receptor that binds to and covers up RANKL molecules sticking out of the surfaces of osteoblastic stromal cells. This prevents the osteoclast progenitors from binding their real RANK receptors to the RANKL on the osteoblastic stromal cells' surfaces, thus preventing the RANK signals needed to drive differentiation of the osteoclast progenitors into mature osteoclasts (Cenci et al, 2000; Grcevic et al, 2001; Whitfield, 2001).

OPG is a member of the TNF receptor family that acts by preventing the association of OPG ligand [OPGL, also known as RANKL, TRANCE, or osteoclast differentiating factor (ODF) with the RANK receptor on osteoclasts and osteoclast precursors. By blocking OPGL/RANKL-induced RANK activation, OPG inhibits osteoclast differentiation, activation, and survival (Grcevic et al, 2001; Khosla, 2001; Kostenuik et al, 2001).

The identification of the OPG/RANKL/RANK system as the dominant, final mediator of osteoclastogenesis represents a major advance in bone biology. It ended a long-standing search for the specific factor produced by preosteoblastic/stromal cells that was both necessary and sufficient for osteoclast development. The initial cloning and characterization of OPG as a soluble, decoy receptor belonging to the TNF receptor superfamily was the first step that eventually led to an unraveling of this system. Soon thereafter, the molecule blocked by OPG, initially called OPG-ligand/osteoclast differentiating factor (ODF) and subsequently RANKL, was identified as the key mediator of osteoclastogenesis in both a membrane-bound form expressed on preosteoblastic/stromal cells as well as a soluble form. RANKL, in turn, was shown to bind its receptor, RANK, on osteoclast lineage cells. The decisive role played by these factors in regulating bone metabolism was demonstrated by the findings of extremes of skeletal phenotypes (osteoporosis *vs.* osteopetrosis) in mice with altered expression of these molecules. Over the past several years, work has focused on identifying the factors regulating this system, the signaling mechanisms involved in the RANKL/RANK pathway, and finally, potential alterations in this system in metabolic bone disorders, from the extremely common (*i.e.* postmenopausal osteoporosis) to the rare (*i.e.* familial expansile osteolysis) (Grcevic et al, 2001; Khosla, 2001; Kostenuik et al, 2001).

Interactions Between Immune System, Estrogen, and Bone Cells

Steroids, and particularly estrogens, are powerful regulators of bone mass. Among other things, they are involved in the differentiation of the B lymphocyte hematopoietic lineage. B lymphocyte precursors (mainly the IL-7 responsive population of the pro- and pre-B cell stages) declined dramatically in the bone marrow of pregnant or estrogen-treated mice. Reciprocally, the same populations increased in hypo-gonadal, ovariectomized or male castrate mice. Increased B lymphopoiesis due to estrogen deficiency and its involvement in stimulating bone resorption was postulated by another group, which investigated the roles of estrogen and estrogen agonists on bone loss. They found that IL-7 receptor knockout mice, which lack mature B lymphocytes due to an arrest at the pro-B stage, have increased trabecular bone volume. Investigating the roles of ovariectomy and orchidectomy on bone loss, Onoe et al suggested possible roles of different cytokines (IL-1, IL-6) and RANKL in the bone loss caused by increased B lymphocyte numbers. Kanematsu et al recently proposed that estrogen deficiency results in an increased production of prostaglandin E2 by osteoblastic and stromal cells, largely resulting from the induction of cyclooxygenase-2 expression by IL-1 and TNF-α. In consequence, the increase in prostaglandin E2 production induces the expression of RANKL on both pre-B cells and stromal cells, which, in turn, leads to accelerated osteoclastogenesis through interactions of RANKL with RANK (receptor activator of nuclear factor-κ-B) on osteoclast progenitors (Grcevic et al, 2001; Kanematsu et al, 2000; Onoe et al, 2000).

The expression of RANKL within the immune system is restricted to T lymphocytes activated by antigen-receptor engagement. RANKL promotes the survival of dendritic cells, their allostimulatory capacity to activate naive T lymphocyte proliferation, and cytokine-activated T lymphocyte growth. In addition, binding of RANKL to RANK regulates T and B lymphocyte development, as well as thymus and lymph node organogenesis, and mammary gland development. Following antigen receptor-engagement, activated T lymphocytes produce membrane-bound and soluble forms of RANKL, and both support osteoclast development *in vivo* and *in vitro*. RANKL-mediated regulation of the skeleton, which impacts both chondrocyte differentiation and osteoclast formation, requires local delivery of RANKL, possibly through enzyme induced shedding of RANKL. It has been suggested that OPG exists in both a membrane-bound and soluble form, and that its expression in B lymphocytes, dendritic cells, and follicular dendritic

cells appears to be upregulated by CD40 stimulation. Some studies havereported that OPG is involved in the regulation of B lymphocyte maturation, dendritic cell stimulatory capacity, and isotype switching during the primary immune response. It has been proposed that OPG acts as a "molecular brake" in RANKL/RANK-mediated immune response, in a manner that is similar to its effect on bone metabolism (Grcevic et al, 2001; Whitfield, 2001).

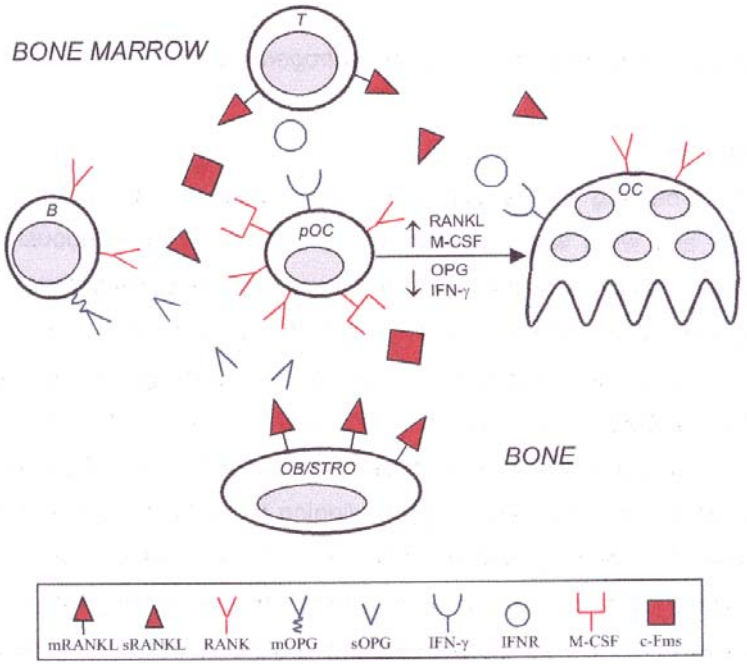

Figure 2. The RANK/RANKL/OPG system interactions between bone and immune cells within the bone marrow microenvirontment. The osteoclast formation from hematopoietic progenitors requires receptor activator of nuclear factor kappa-B ligand (RANKL) and macrophage colony-stimulating factor (M-CSF). Both of these molecules are produced by osteoblastic/stromal calls membrane-bound RANKL, attaches to RANK receptor and soluble M_CSF binds to its receptor molecule c-Fms on osteoclast progenitors. The osteoblastic/stromal calls also produce the soluble dewy receptor osteoprotegerin (OPG), which binds to RANKL and acts as a molecular inhibitor between RANK/RANKL interaction. The same molecules exist within the immune system. Activated T-Lymphocytes produce RANKL. which interacts with RANK and OPG expressed on B-Lymphocytes, stimulated by CD-40 ligation. At the same time, T lymphocyte produced mebrane-bound and soluble RANKL, as well as M-CSF, promote osteoclastogenesis. In addition, T lymphocytes secrete IFN-gamma, which serves as a negative regulator of osteoclastogenesis. Binding of IFN-gamma to its receptor interferes with RANKL/RANK system by disruption of signal transduction. Thus, both osteoblastic/stromal cells and activated T lymphocytes are stole to regulate osleoclastogenesis by producing both essential stimulators (RANKL and M_CSF) and an inhibitor (OPG or IFN-gamma respectively). The question is if them are other molecular interactions between bone and immune cells in the bone marrow microenvironment. T - T lymphocyte; 8 - B lymphocyte; pOC - osteoclast precursor, OC - osteoclast; OB/Stro- osteoblastic/stromal cells; IFN-gamma - interferon gamma; IFNR - IFgamma-receptor. RANK - receptor activator of nuclear factor kappa-B; RANKL - RANK ligand; OPG - osteoprotegerin; M_CSF - macrophage colony stimulating factor, c-Fms = M_CSF receptor; s - soluble form; m - membrane-bound form; upregulation; 4 - downregulation (Grcevic at al, 2001).

When estrogen falls below a critical level, all of these restraints on osteoclast production are lifted (Figure 3). Only the amount of bone a woman has banked during her youth will determine whether she will be at risk for osteopenia and be extra susceptible to traumatic fracture or osteoporosis and associated with "spontaneous" fracture. The IL-1beta level in the stromal microenvironment rises, and the IL-1beta decoy receptors disappear, leaving the way open for the surging interleukin to stimulate monocytes and T cells to make TNF-alpha, which in turn stimulates osteoclast generation (Figure 3). TGF-beta production by

osteoblastic stromal cells also falls off, and with it osteoclast apoptosis, and their OPG/RANKL production ratio shifts in favor of RANKL, while the production of the osteoclast-stimulating M-CSF, IL-6, and IL-11 rises (Figure 3) (Cenci et al, 2000; Grcevic et al, 2001; Whitfield, 2001).

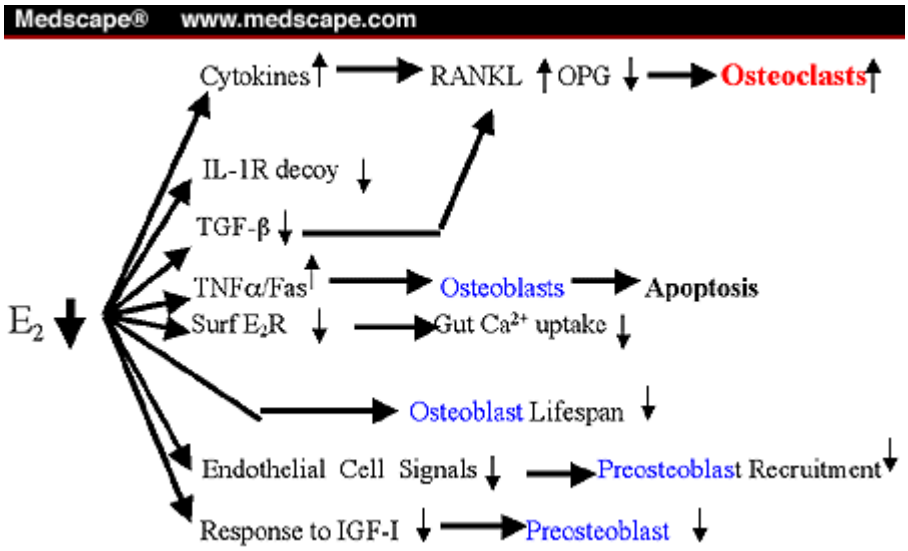

Figure 3. What happens to stimulate osteoclast generation and increase bone turnover when estrogen (E_2, 17beta-estradiol) falls below a critical level. A drop is designated by ↓ and a rise by ↑. These events are discussed in the text. "Surf E_2R" refers to "nongenomic" estrogen receptors fixed on the cell surface; "IL-1R decoy" refers to the IL-1 decoy receptor (Whitfield, 2001).

These events result in an osteoclast population explosion. More BMUs with larger osteoclast crews appear. Bone turnover accelerates and the deeper-digging osteoclasts increase the remodeling space. The thinning and porosity of the hip cortex and the destruction of the trabecular lattice of the vertebrae escalate dangerously. Osteoblasts are not unaffected by the decline in estrogen level with menopause. Estrogen increases the working lifespan of preosteoblasts and osteoblasts, perhaps at least in part by preventing them from responding suicidally to the large, apoptosis-triggering amount of phosphate, which they meet in osteoclast excavations. But the increased IL-1beta and TNF-alpha production resulting from the estrogen drop increases the likelihood of osteoblastic cells responding to the high phosphate levels in osteoclast excavations by increasing their *Fas* expression and killing themselves by Fas protein-triggered apoptosis (Figure 3). Intestinal cells also have "nongenomic" estrogen receptors fixed to their surfaces in addition to the "genomic" receptors that operate in the nucleus to modulate the expression of various genes. The fading of nongenomic receptor signaling with menopause may further reduce the uptake of dietary Ca^{2+} and increase the bone loss due to the vitamin D deficiency that commonly develops in older people. Adding to this vitamin deficiency is a decrease in the estrogen-dependent expression of the 1alpha,25-dihydroxyvitamin D_3 receptor and the 1alpha,25-dihydroxyvitamin D_3-dependent calbindin-9kDA protein that carries Ca^{2+} across the intestinal epithelial cells and dumps it into the blood (Figure 3) (Jilka et al, 2001; Roach, 2000; Whitfield, 2001).

From many studies above, the author made a simplification chart about the interactions between estrogen; immune system; and bone cells (Figure 4). Estrogen facilitates expression of IL-1 β type 2, which function as a decoy to decrease the production of TNF-α, respectively osteoclastogenesis. Estrogen also inhibits maturation of pre-lymphocyte B in bone marrow. Estrogen can prevent RANK/RANKL system by increasing the production of OPG as a blockade receptor, so osteoclastogenesis decline. And finally, estrogen limits the size of preosteoclast and osteoclast population by a transforming growth factor (TGF)-beta-mediated killing of these cells by apoptosis. The end result is, estrogen prevent bone loss by influencing the immune system interactions with bone through several related cytokines from both systems.

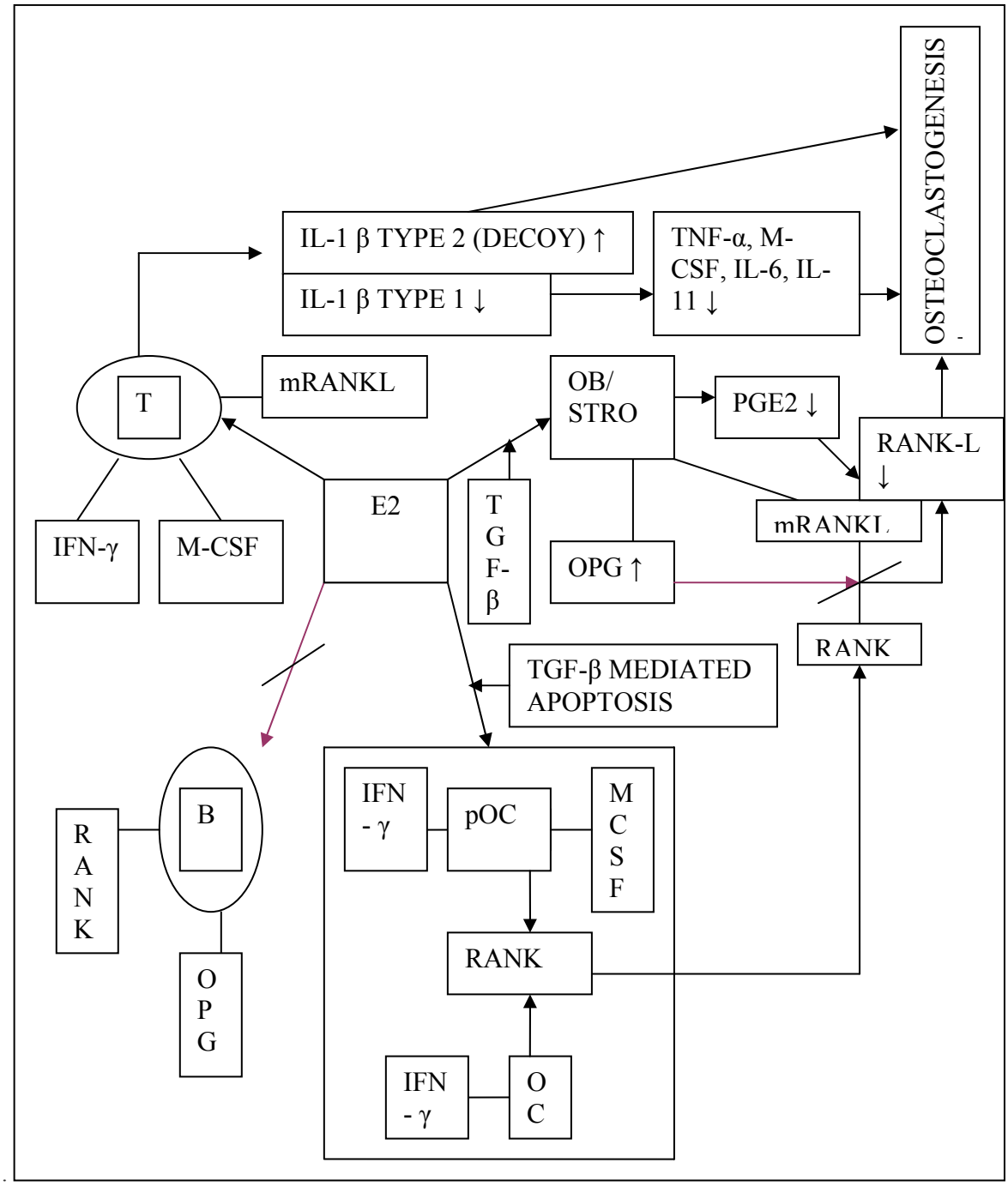

Figure 4. Role of Estrogen in Interaction Between Immune System and Bone Cells. E2 – Estrogen, T – T lymphocyte, B – B lymphocyte, OB/STRO – steoblastic/Stromal Cell, pOC – Osteoclast precursor, OC – Osteoclast, inhibit / blocking facilitating producing (Kalanjati, 2005).

CONCLUSION

Estrogen affect both cells from immune system and bone producing varieties in bone turnover. Emerging from the changes unleashed by the menopausal estrogen decline are an increased number of BMUs with bigger osteoclasts and smaller, shorter-lived osteoblast crews, an expanding resorption space, and weaker, more porous bone. Those can be happened when RANK/RANKL system engaged so bone turnover

increase with larger remodeling spaces and wider trabecular destruction. OPG can block RANK/RANKL engagement by being a soluble, decoy receptor. In the future, the author suggest the readers of this review to conduct more studies related with this subject to get better solutions for osteoporosis treatment.

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