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Do Cytokines Portray Distinct Osteoimmunology?-Establishing the Unique Role.

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ABSTRACT

Osteoimmunology is an interdisciplinary field where an extensive reciprocal interaction between the immune and skeletal system occurs. Such a discovery has been made almost 40 years ago. This mutuality was well established after the discovery and characterisation of the novel signalling pathway, known as RANKL/RANK -OPG pathway, which are the members of tumor necrosis factor (TNF) and TNF receptor super families. This review summarizes the different molecular mechanisms taking place at the cellular level for osteoclast formation and interactions of cytokines on this new signalling pathway. However, some pro-inflammatory cytokines such as IL-2,5,9,14,16,19,20,22,24,25,26,28 etc, still require extensive research for its action on RANK/RANKL-OPG mechanism.

Keywords: Bone remodelling, OPG, Osteoclastogenesis, RANK, RANKL

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INTRODUCTION

Osteoimmunology is primarily concerned with the mutual interaction between the skeletal and immune system. The term osteoimmunology has been originally coined by Arron and Choi Y [1], in order to look closely into the actual reciprocity between skeleton and the immune system. A critical turning point in osteoimmunology happened with Penninger and his colleagues [2]. These pioneers have established for the first time the role of the RANKL\RANK system in bone disorders. Subsequently, osteoimmunoendocrinology emerged as a new development in osteoimmunology [3].

Both the bone and the immune system share a large number of regulatory cytokines and other molecules in common. Structurally, cytokines are polypeptides, acting as the communication intermediaries between the cells, which can give command for proliferation and differentiation [4]. The most important group of cytokines known today are Interleukins [IL]. As the term itself implies, these cytokines remain in constant communication with leukocytes. The data available till date indicate that there are 38 ILs which are labelled from IL-1 to IL-38.

Normal bone remodelling mechanism

In the medical terminology, bone is stereotyped as a protective and supportive framework of the human body. The most important tasks of the bone include protection of the internal organs, enabling bodily movements, storing life-saving minerals such as calcium and phosphorus, helping as an energy reservoir and, last but not least, cooperate in the production of blood cell, particularly in bone marrow. Throughout the entire human life, bone undergoes continuous remodelling, which involves bone resorption and deposition [5]. This mechanism was first observed by Julius Wolff, a world famous researcher, over 100 years ago. The four processes involved in this functional adaptation are recognized as activation, resorption, reversal and formation [6]. It is assumed that the human bone tissues are constantly replaced with new ones every 10 to 25 years. Such a rejuvenation is needed for withstanding stresses occurring in the day to-day life.

The basic multicellular units in cortical and trabecular bones are the main sites where the coupling process of osteoblasts and osteoclasts usually takes place [7]. Therefore, the major components in bone remodelling are osteoblasts and osteoclasts. The cuboidal osteoblasts, which sprout from osteoprogenitors contribute substantially in the formation of osteoid and in mineral deposition. The osteoclasts are large polykaryons, usually containing 5-8 nuclei, but in pathological conditions the number can go up to even one hundred or more. It also contains a reasonably high number of mitochondria. It usually has a life span of two weeks [8, 10]. The discovery of osteoclasts and its detailed morphological features in 1873 by Albert Kolliker [9] in Germany was certainly an important milestone in the history of bone remodelling. Disproportionate bone remodelling can result from various conditions such as hormonal changes and increased generation of inflammatory cytokines wherein the outcome would be an increase or decrease in bone mineral density. In normal and pathological conditions, bone resorption or formation occurs mainly in trabecular bone. It is a well-established fact that bone remodelling can occur in each individual annually in up to 10 % of the entire system [10, 11].

During bone functional adaptation, the osteoclasts originate from hematopoietic mononuclear precursor cells. It resorbs bone by the release of acid and proteolytic enzymes such as cathepsin K in the resorption lacuna. As a next step, preosteoblasts move in, and differentiate into osteoblasts, which subsequently produce osteoid, composed of collagen, osteonectin and other proteins which mature and then mineralize during the following period [12].

Molecular mechanism of osteoclastogenesis

Medical science suggests that most of the bone ailments in humans are characterised by a process known as bone resorption. An inflammation always transmogrifies resorption of bone usually through three important processes. First and foremost, the pro-inflammatory cytokines modulate the osteoclast function by means of a Receptor Activator of the nuclear factor-κB (RANK) and its functional ligand (RANKL) - also known as TNF related activation induced cytokine (TRANCE). Secondly, it happens through the crucial role of Macrophage colony stimulating factor (M-CSF) and, thirdly, through transcription factor, PU.1. M-CSF passes its osteoclastogenic signals to the cell through the receptor c-fms. They further stimulate the expression of

RANK in monocyte-macrophage precursor cells. The transcription factor PU.1 actually regulates the development of osteoclasts by controlling the expression of c-fms [13].

The major pathways of osteoclast differentiation are pictorially depicted in Figure 1. In one of these pathways, the RANKL/RANK signalling cascade induces osteoclastogenesis, which is commenced by the activation of TNF receptor-associated factor 6 (TRAF6), a pathway described by the pioneers Armstrong et al [14]. The two most common downstream mediators in this pathway are nuclear factor kappa-light-chain-enhancer of activated B cells (NF- κB) and activated protein 1 (AP-1). Several co-stimulatory molecules are present in the other pathway such as osteoclast associated receptor(OSCAR),paired immunoglobulin-like receptor A (PIR-A), signal-regulatory protein β (SIRPβ) and triggering receptor expressed on myeloid cells (TREM2) which are essential factors for osteoclast activation. They initiate Ca^{2+} signalling cascade through immunoreceptor tyrosine-based activation motifs (ITAM) phosphorylation. The Ca^{2+} signalling cascade along with the action of transcription factors such as NF-κB and c-fos result in the auto amplification of nuclear factor of activated T cells cytoplasmic1 (NFATc1), which is the master regulator of osteoclastogenesis [15,16].

A novel signalling pathway

The discovery of RANKL/RANK-Osteoprotegerin (OPG) system can be considered as a quantum leap in the understanding of bone biology. It was first identified in the 1990s. In 1997, Simonet et al [17] discovered for the first time OPG as an inhibitor of osteoclastogenesis. It was followed by the discovery of RANKL in 1998 as an inducer of osteoclastogenesis. The interruption in this system is responsible for a disrupted bone homeostasis. The newly found cytokine RANKL is the corner stone in the osteoclast generation and its activity. RANKL, RANK and OPG are pivotal regulators of bone metabolism belonging to Tumour Necrosis Factor receptor and ligand families. RANKL, a type 2 ligand protein of TNF ligand family is an essential instigator for osteoclast differentiation. This factor exists as a homotrimeric protein and is expressed as a membrane - bound protein on the surface of osteoblasts, osteocytes, marrow stromal cells, activated T cells, and B-cells. M-CSF, along with most osteotropic factors such as IL-1, IL-11, prostaglandin E2 and 1,25-(OH)2D3, induces osteoclast formation by binding to marrow stromal cells, which in turn express increased levels of soluble or membrane forms of RANKL [18].

RANKL, also known as Osteoclast Differentiation Factor, is produced by osteoblasts, marrow stromal cells and activated T lymphocytes. RANKL can interact with the two receptors which have antagonistic type of action on osteoclast differentiation. RANK is a heterotrimeric transmembrane protein member of the TNF receptor superfamily that appears to be expressed in osteoclast precursors, mature osteoclasts, dendritic cells, mammary glands and some cancer cells, like breast and prostate cancers. RANKL binds to its receptor RANK, expressed by hematopoietic cells and thereby initiates osteoclast development. This action is indispensable for osteoclast differentiation, function and survival (Figure 2) [19, 20]

OPG, which is a secreted member of TNF receptor family, is produced by osteoblasts, marrow stromal cells similar to RANKL molecule. Its main action is to inhibit osteoclast formation and activity. OPG, the name itself signifies protector of bone. It is the first molecule of the 3 different key players that can control osteoclast function. Also known as osteoclastogenesis inhibitor factor, it is a soluble protein secreted by osteoblasts, 64% of which is produced by B-cells. OPG production is controlled by cytokines, growth factors, hormones and Wnt/beta-catenin. OPG acts as a soluble inhibitor of osteoclast maturation and osteoclast activation *in vitro* and *in vivo*. In the RANKL-RANK-OPG mechanism (Figure 2), OPG, binds with RANKL hindering further interaction of RANKL with RANK. This process inhibits the differentiation of preosteoclasts into mature osteoclasts [21, 22].

Cytokines in Bone Remodelling

Cytokines

The term 'cytokine' was coined by Cohen et al. in 1974, prior to which, it was called 'lymphokine' which meant it was derived from activated lymphocytes, particularly, the Th cells. According to Ceciliani et al. [23], cytokines are small secreted proteins that enhance the interaction and communication between cells. Based on the process of their origin, cytokines are classified into lymphokines, monokines, chemokines and interleukins.

Ozaki and Leonard in 2012 [24] have discovered that ILs, Interferons (IFN) and Tumour Necrosis Factor (TNF) are the most outstanding cytokines. The ILs that enhance the bone resorption are IL-1, IL-3, IL-6, IL-12, IL-15 and IL-23, particularly, in diseases like rheumatoid arthritis and periodontitis. Several of these compounds have been shown to increase RANKL expression. Among the ILs, IL-4 and IL-13 inhibit bone resorption and IL-1, IL-11 to 19, TNF-α, IL-6, IL-20 to 25 are known as the pro-inflammatory cytokines proficient in osteoclastic bone resorption [25].

Cytokines with the RANKL/RANK-OPG mechanism

IL-1

IL-1 is a polypeptide, with the predominant molecular weight of 17kD. The two distinct forms of IL-1 are IL-1α and IL-1β. While most of the activity in circulation comes from IL-1β, IL-1α is always membrane associated. Following lipopolysaccharide stimulation, the intracellular activity and extracellular activity are detected in 30 min and 60 min respectively. Two types of receptors are found, namely, an 80kD (type 1) and a 60-70kD (type 2). Type 1 IL-1 receptors (IL-1RI) are found in almost all cells, while type 2 IL-1 receptors (IL-1RII) are active in B-cells [26].

The IL-1 cytokine family comprises 10 members. IL-1 is secreted by activated macrophages and neutrophils. Previously, it was known as an 'endogenous pyrogen', and later, as a lymphocyte activating factor. The major functions of IL-1 centre around stimulating the activation of Th cells, which in turn help the maturation and clonal expansion of B-cells [27]. It has a positive impact on the expression of RANKL and RANK on cells [28].

IL-1 is a pro-inflammatory cytokine that can lead to bone havoc in certain ailments such as osteoporosis, rheumatoid arthritis and periodontal diseases. The mechanism of IL-1 induced bone resorption in the above mentioned pathological conditions is that it stimulates osteoclast differentiation, fusion, and activation by activating the early signalling pathways of NF-κB, JNK and p38. All these are exceptional processes for RANKL induced osteoclast differentiation. The main target of IL-1 is on mature osteoclasts. It can directly instigate bone resorption through the IL-1RI receptor. Furthermore, the count of the IL-1RI receptor increases by the osteoclast-inducing factors such as RANKL and TNFα- via c-Fos and NFATc1. Alternatively, IL-1 can directly induce osteoclastogenesis through the activation of specific genes, including TRAP and OSCAR, in part via the microphthalmia-associated transcription factor (MITF) pathway [29]. IL-1β is always associated with increased OPG [30].

IL-3

The actual sources of this cytokine are Th1, Th2 cells and macrophages.IL-3, also known as multilineage-colony-stimulating factor, stimulates the growth of mast cells and promotes the development of hematopoietic progenitor cells into neutrophils, macrophages, megakaryocytes and mast cells. It acts as the connecting link between the immune system and hematopoietic system [31, 32].

IL-3 also has an inhibitory effect on osteoclast differentiation mediated by RANKL and TNFα, by direct action on early osteoclast precursors. On the other hand, it has no inhibitory effect on mature osteoclasts. Moreover, the inhibitory effect of this cytokine is irreversible [33].

IL-4

IL-4 is a pleiotropic cytokine produced by Th2 lymphocytes, mast cells, and eosinophils. The level of the cytokine surges during chronic inflammatory conditions. This cytokine is secreted by CD4+Th2 cell and it is an autocrine growth factor for CD4+Th2 cell. It causes activation, proliferation and differentiation of B- cells. It inhibits IFN-γ induced macrophage activation and thereby restrains cell mediated immunity. The previous report by Abu-Amer [34] elucidates that IL-4 diminishes the RANK mediated osteoclastogenesis via inhibition of NF-κB activation. Furthermore, it is stated that the main action of IL-4 is to activate the STAT 6 dependent mechanism, which acts as an NF-κB inhibitor [35]. This entity is associated with increased OPG, which is a bone protective factor [36].

IL-6

IL-6, which was first identified in 1986, is an osteoclastogenic inflammatory cytokine mainly produced by macrophages, osteoblasts, osteoclasts and activated T-cells in response to IL-1 and TNF- α. By the year 2005, Kishimoto [37] discovered the association between cytokines in various complex functions such as the regulation of hematopoiesis in bone marrow, maturation and activation of B-cells, along with T-cells, osteoblasts, osteoclasts, chondrocytes and endothelial cells. Shaama et al. reported in 2005 [38] that IL-6 induces osteoclastogenesis which is exactly similar like IL-3, IL-11 and Tumour necrosis factor. Furthermore, IL-6 was found to inhibit function of human osteoblasts.

The research by Yoshitake et al.in 2008 [39] proved beyond doubt the involvement of IL-6 in RANK signalling pathways, particularly in stimulating osteoblast proliferation and differentiation. IL-6 has been shown to stimulate both RANKL and OPG production in bone via RANKL dependent and RANKL independent mechanisms. Kim et al. [40] indicated that inflammation results in release of unusual number of osteotropic cytokines such as IL-1, IL-6, IL-11, which in turn enhance the expression of TRANCE through activated osteoblasts, T–cells, marrow stromal cells and mammary gland epithelial cells. The TRANCE thus generated further leads to osteoclastogenesis. This cytokine may exert its inhibitory effect on bone formation directly through gp130-STAT 1/3, indirectly with its influence on the balance between OPG, RANK and RANKL [41].

IL-7

IL-7 is a critical cytokine required for the early development and expansion of precursor B and T cells. It is also a potent stimulator of immature B Cells. Stromal cells in bone marrow promote the development of B-cell, these cells in turn produce IL-7. It is a direct inhibitor of osteoclast formation [42]. On the contrary, it is also demonstrated that systemic administration of IL-7 increases osteoclastogenesis in human peripheral blood cells, through the upregulation of osteoclastogenic cytokine production in T-cells [43]. It further increases the T-cell production of RANKL, and is also associated with increased OPG. In the bone marrow, the production of IL-7 in osteoblasts is essential for normal development of B-lymphocytes. During early development, B-cells express RANKL. Lee et al. stated that there is an interconnection between RANKL and IL-7 [42].

IL-8

The sources of IL- 8 are known to be macrophages, neutrophils and endothelial cells. Originally, it acts as the chemo attractant for neutrophils in inflammation. It stimulates bone resorption and osteoclast formation by a mechanism independent of RANKL pathway [44]. It stimulates the activity of mature osteoclasts. In bone, the sources of IL-8 are osteoblasts and osteoclasts. Other sources are bone marrow stromal cells, synovial fibroblasts and chondrocytes. The presence of IL -8 is increased in medical conditions such as osteoarthritis, temporomandibular joint disorders, periodontitis and osteomyelitis. In all these conditions, osteoclast activation leads to destruction of bone and joints. IL-8 can directly foster both osteoclastogenesis and osteoclast-mediated bone destruction [45].

IL-10

Earlier IL-10 was described as a cytokine produced by Th2 cells. The major part of this cytokine is secreted by macrophages, and to a lesser extent by dendritic cells. It turns down activated macrophages and dendritic cells and has control over innate as well as adaptive immunity [46]. It is often referred to as cytokine-synthesis-inhibitory factor. It actually is an inhibitor of Th1 cytokine production, a process responsible for inhibition of pro-inflammatory cytokines and IL-12 production.

A very recent study by Liu et al. proved that Interleukin-10 enhanced the expression of OPG and down-regulated the expression of RANKL. Thus, this cytokine is associated with bone protective function [47].Interestingly, it inhibits osteoclast formation in rat by directly acting on hematopoietic osteoclast precursors [48].The main target cell of this cytokine in the regulation of osteoclast is osteoclast precursor cells.

IL-11

IL-11, as a member of the interleukin-6 family of cytokines, can play a critical role in osteoclast development and activation. As demonstrated by Girasole et al [49], the main function of IL-11 is not only to enhance mineral mobilization, but also to increase bone matrix degradation. The research carried out by Ahlen et al. [50] suggests that IL-11 stimulates bone resorption in mouse calvarias by enhancing the number of osteoclasts and it is known as a factor associated with increased RANKL and OPG expression.

IL-12

IL-12 is the prototype member of a small family of heterodimeric cytokines, which also includes IL-23 and IL-27. IL-12 is actually generated by macrophages and dendritic cells and has been shown to potently induce the production of IFN-γ by T and NK cells. IL-12, also referred to as NK cell stimulatory factor, is the jump-starter of cell mediated immunity and operates as the link between innate and adaptive immunity systems. The receptor of IL-12 comprises 2 chains, namely, IL-12Rβ1 and IL-12Rβ2. These receptors are primarily expressed on activated T-cells and NK- cells [51].

IL-12, as a potent pro-inflammatory cytokine, is a fundamental regulator of the immune response to antigenic challenge that promotes Th1 and at the same time suppresses the Th2 cell response. IL-12 is a strong inhibitor of osteoclast formation, whereas IFN-γ is a mediator of the inhibitory effects of IL-12 [52].

IL-13

IL-13, a 10kDa protein secreted by Th2-type lymphocyte, inhibits bone resorption by increasing the level of OPG and decreasing the level of RANKL and RANK in a STAT6 dependent pathway by activating receptors on osteoblasts and osteoclasts that affect the RANKL/RANK/OPG system [53]. It also inhibits bone resorption by restraining prostaglandin synthesis. The molecular mechanism behind this is down regulation of the mRNA expression of cyclooxygenase-2 in osteoblasts [54].

IL-15

IL-15 is secreted by B- cell, T cell, NK cell, monocytes and sometimes by macrophages also.].It is a member of IL-2 family, and therefore its functions are similar to IL-2.It also helps in the proliferation of T, B and NK cells, acts as a specific chemo attractant for T-cell, and finally helps in the cytokine production in NK and CD8+T cells. Ogata et al mentioned in their study that this cytokine can increase the count of osteoclast progenitor cells in the culture that in turn leads to increase in bone resorption [55].Moreover, in rheumatoid arthritis patients the mechanism of osteoclastogenesis is stimulate by the action of IL-15 [56].

IL-17

The term Interleukin-17 (IL-17) was first used in 1995 by Yao et al [57].Later, in 1999 it was reported by Kotak et al. that IL-17 potentially stimulates osteoclastogenesis through the expression of RANKL on osteoblasts [58].

IL-17, a well-known pro-inflammatory cytokine is produced by memory T-cells. There are 6 members of IL-17 cytokine family, namely, IL-17A, IL-17B, IL-17C, IL-17D, IL-17E and IL-17F. Van Bezooijen [59] suggested in 1999 that the receptors for IL-17 are present in osteoblasts and thus show their signature role in bone metabolism. Later in 2012, Moon et al. [60] pointed out the cardinal role of IL-17 in bone remodelling, particularly in enhancing osteogenesis. They also stated that rheumatoid arthritis triggers a remarkable increase in IL-17 production leading to osteoclastogenesis in bone microenvironment.

In 2014, Chen et al. [61] detected a copious secretion of IL-17 in osteoarthritis patients, and that resulted in IL-17 being established as an exceptional marker for diagnosing osteoarthritis. Koenders et al. [62] made a surprise finding in 2005, through his strictly experimental study, that a remission was seen in joint inflammation and bone erosion soon after the blocking of IL-17 via the inhibition of RANKL and IL-1 in osteoarthritis. Another study by Nam et al. in 2012 [63] indicated that osteoblast regulation was intensified by IL-17 during the initial stage of fracture repair and bolstered osteogenesis along with IL-6.

The pro-inflammatory cytokine IL-17 stimulates the production of IL-1β, IL-6, IL-8 and G-CSF. It is secreted by T- cells. IL-17 probably exerts its osteoclastogenic effects by stimulating RANKL expression on osteoclastogenesis-supporting mesenchymal cells such as synovial fibroblasts and osteoblasts, while concurrently activating local inflammation, leading to the release of inflammatory cytokines, such as IL-1, IL-6 and TNF. These cytokines additionally increase RANKL expression on osteoclastogenesis supporting cells and enhance RANKL signal transduction in osteoclast precursor cells, synergistically promoting osteoclastogenesis in the inflamed synovium [64].

Activated T helper cells that secrete IL-17 in abundance has been termed Th17. IL-23 is the cytokine which supports T – cells production of IL-17, thus promoting osteoclastogenesis. The study postulated by Sato et al. [65] mentioned that IL-23 - IL-17 axis is essential for onset and destruction phase of autoimmune arthritis characterized by T-cell mediated activation of osteoclastogenesis. The inflammatory cytokines, such as IL-17, IL-1β, IL-6 and TNF-α, foster RANKL expression not only on osteoblastic stromal cells, but also on osteoblasts and bone marrow stromal cells. Several studies proved that IL-17 facilitates bone loss by regulating the RANKL/OPG ratio. It impedes the expression of OPG, thereby facilitating bone resorption. Thus, it is established that in osteoblastic cells IL-17 increases RANKL expression, thereby leading to bone erosion *in vitro* and *in vivo* [66].

IL-18

The dendritic cells, and in rare situations macrophages, produce IL-18 that induces IFN- γ production by NK and T- cells. It is functionally similar to IL-12 and structurally related to IL-1β. It enhances NK cells and T-cells maturation, cytotoxicity and cytokine production. Horwood et al. [67] stated that the combined action of IL-18 and IL -12 inhibits bone resorbing mechanism of osteoclast. IL -18 induces the production of chemokines, IL -6 and IL-8. It stimulates Th1 responses in combination with IL-12.In combination with IL-2, it also stimulates Th2 responses. In contrast, IL-18 may have a protective effect on bone erosions. Osteoblastic stromal cells produce IL-18, which inhibits osteoclast formation, apparently through the release of GM-CSF by T cells.

In bone, IL-18 is mainly produced by macrophages and osteoblasts, and it prevents osteoclast formation through granulocyte-macrophage colony-stimulating factor (GM-CSF) and, not IFN -γ production by T cells. GM-CSF is a known autocrine mitogenic factor for osteoblasts. OPG has also been recognised as an IL-18 target gene in bone marrow stromal ST2 cells. The expression of OPG mRNA was increased, reaching a maximum level 3 h after IL-18 exposure. IL-18 does not affect bone resorption. Further, IL-18 acts in association with IL-12 to inhibit osteoclast formation. It also plays a key role in T cell development and activation [68].

IL-23

IL-23 is a pro-inflammatory cytokine which promotes the differentiation of Th17 [69]. Th17 cells are the main source for cytokine IL-17A. It induces production of cytokines such as IL-1, IL-6 and TNF by macrophages. IL-17A fosters the production of RANKL by osteoblasts and upregulates RANK on osteoclast precursors. Recent studies point to the role of IL-23 which is involved in osteoclastogenesis, independently from IL-17, via induction of RANKL expression [70]. Moreover, IL-23 inhibits osteoclast formation indirectly via T cells *in vitro* [71].

IL-27

IL-27 belongs to the long-chain 4-helix bundle family of proteins. The dendritic cells and monocytes produce cytokines, which induce Th1 response and enhance the production of IFN γ. IL-27 is not only involved in directing the propagation of naive T cells, but also in the proliferative response and the cytokine production of antigen-specific effector/memory Th1 cells. Kamiya et al. [72] in their studies enlighten that IL-27 has the potential to prevent RANKL expression on osteoblasts stimulated by IL-6. They also demonstrated that this cytokine could partly modify cell fusion or the survival of multinucleated osteoclasts. In these studies, they also found an inhibitory role of this cytokine in osteoclast differentiation.

IL-32

Interleukin-32 (IL-32) is a novel cytokine which has the typical properties of a pro-inflammatory mediator by stimulating TNF-α, IL-1β and IL-8 production. It activates the NF-κB and p38 mitogen activated protein (MAP) kinase pathways. The key sources of IL-32 are mainly T- cells, natural killer, epithelial cells and monocytes after stimulation by IL-2 and IL-18. The induction of NFATc1 is essential for osteoclast differentiation. IL-32 always acts by upregulating the expression of NFATc1 which is independent of the RANK/RANKL pathway. IL-32 can also stimulate osteoclastogenesis. However, it has the disadvantage that it cannot further activate this newly formed multinucleated cells into bone resorbing osteoclasts. IL-32 could also indirectly modulate osteoclastogenesis *in vivo* [73].

IL -33

IL -33, a new member of IL -1 family, increases production of IL-4, IL-5, and IL-13 by polarized Th2 cells. Furthermore, it increases not only production of IL-4, IL-5, and IL-13 by antigen-stimulated splenocytes and chemo attraction of polarized Th2 cells, but also of IL-6, IL-1β,TNF-α, and PGD2 by bone-derived mast cells. The synergic effect with IgE cross-linking on production of IL-6 and IL-13 by bone-derived mast cells increases production of Th2 cytokines and chemokines by human peripheral blood and cord blood-derived mast cells [26]. It helps also in the treatment of bone resorption, since it directly inhibits RANKL mediated osteoclast differentiation, through it's dual-function, namely, as an intracellular, as well as mediating pro-inflammatory response as an extracellular cytokine [74]. Keller et al. [75] illustrated that the over secretion of IL-33 in osteoblasts can cause reduction in osteoclastogenesis *in vivo*.

IL -34

IL-34 gets directly involved in the destruction of bone by increasing osteoclast activation and proliferation as evidenced in cases of rheumatoid arthritis. It was recognised way back in 2008, that IL-34 promotes macrophage colony formation, almost the same way as M-CSF [76]. It maintains monocyte survival and proliferation, but at the same time prevents its differentiation to macrophages. This interleukin has a crucial role to play in osteoclastogenesis, as it induces proliferation and adhesion of osteoclast progenitors in the presence of RANKL *in vitro* [77].

Interferons

It is a well-established fact that the interferons (IFN) have the crucial role in the self-defence-system of the human body. There are two types of IFNs, Type 1 and Type 2. IFNα and IFNβ are part of the type 1 IFNs that emerge from virus infected cells. IFNγ or type 2 interferon, is secreted by the activated T cells and NK cells. Among the different types of IFNs, IFN-γ and IFN -β strongly suppress osteoclastogenesis by inhibiting RANK signalling. IFN-γ inhibits osteoclast formation by the down regulation of osteoclast gene, cathepsin K [78].

The cytokines IL-1, IL-3, IL-6, IL-7, IL- 8, IL-11, IL-15, IL-17, IL-23 shown in Figure 3, activates RANKL, which in turn assists in the conversion of osteoclast precursors to osteoclasts. Figure 3 also illustrates the various cytokines which inhibits the RANKL mechanism. Figure 4 details the various cytokines that can increase or decrease the production of OPG.

Cytokines in which RANKL/RANK-OPG mechanism is unidentified

IL-2

IL-2 is primarily generated by CD4+ Th1 cells and, to a lesser degree by CD8+ Th1 cells. It was originally referred to as T- cell growth factor. It is generally considered to be an autocrine growth factor because it acts on the T- cells which finally produce it. It is not only a growth factor for activated T- cells but also a differentiation molecule that promotes both Tc-cell and B-cell activities. Combined with IL-12, it increases cytotoxicity of NK cells and lymphokine-activated-killer cells. The surge of IL-2 levels in active periodontal

pockets, suggest a destructive role for Th1 responses in the periodontium. So the higher expression of IL-2 levels in periodontium indicates acute form of periodontal disease [79, 80].

IL-5

IL-5 is secreted by CD4+ Th2 cell. The action is same as IL-4 such as B-cell activation, growth and its differentiation as well as the eosinophil differentiation. Its biological mechanism is initiated only after binding itself with its membrane bound IL-5R .In allergic diseases and asthma, there are high levels of IL-5 in circulation along with high levels of eosinophils [81].

IL-9

IL-9 is a pleiotropic cytokine produced by T-lymphocytes. The subset Th17 also has a role to play in IL-9 production. It also acts as the mast cell growth factor and a major inducer of IL-6 production. In addition, it acts in synergy with IL-5 for eosinophil maturation. IL-23 shows an inhibitory role, when it comes to IL-9 production. TGF-β and IL-4 can modulate the production of IL-9. It could further promote the growth of CD4+T cells and Th17 cells, the same way as it enhances B cell development and function. IL-9 synergised with IL-3, further activates the colony forming unit of erythroid [82].

IL-14, IL-16

IL-14 is a novel cytokine, secreted by T-cells, promoting the growth of B-cells, although its functioning could not be well established yet [83].In inflammation, IL-16 eases out the selective movement of CD4 T cells, monocytes and eosinophils. The source is mainly attributed to the Th as well as Tc cells. IL-16 was up-regulated in CD4 T cells in the early phase of the response. IL-16 has been shown to be involved in the selective migration of CD4 T cells, as it participates in the inflammatory diseases [84, 85].

IL-19, IL-20

IL-19 is a 21-kD protein secreted mainly by LPS activated monocytes, and is a novel cytokine of the IL-10 family. It modulates Th1 activity. Sakurai et al. stated that the joint inflammation is promoted in Rheumatoid arthritis (RA) patients by IL-19, produced by synovial cells [86, 87].

IL-20 is generated from monocytes and keratinocytes. It regulates mainly the inflammatory responses of skin [88, 89, 90].

IL-22, IL-24

IL-22 is generated by activated Th1 cells and induces acute phase proteins. It impedes not only the IL-4 production by Th2 cells, but also hinders the reorganization of the wound healing tissues [91, 92].

IL-24 takes its origin from monocyte and T- cells. It induces cytokines such as IL-1, IL-6 and TNF.A previous study concluded that IL-24 is responsible for an increase of synovium in patients with rheumatoid arthritis. IL-24 is further implicated in the recruitment of neutrophil granulocytes [93, 94].

IL-25, IL-26

IL-25 or IL-17E was considered earlier as part of the IL-17 cytokine family, along with IL-17F.The Th2 cells, mast cells and macrophages are the main sources of IL-25. It induces the production of IL-4, IL-5 and IL-13 [95].

IL-26 enhances the production of IL-8 and IL-10 by epithelium. It is generated by T-cells and NK cells. It helps in activation and regulation of the epithelial cells [96].

IL-28, IL-29

The dendritic cells and monocytes produce these cytokines, which obstruct the viral replication and type 1 IFN activity [97, 98]. IL-28 also plays a role in allergic and autoimmune diseases.IL-29 stimulates

monocytes and macrophages to produce certain cytokines. In exposed peripheral blood mononuclear cells and macrophages, IL-29 increases the production of IL-6, IL-8, and IL-10. IL-29 has also been shown to inhibit the production of IL-13 by T cells [97, 98, 99].

IL-30, IL-31

IL-30 is the p28 subunit of IL-27, and is produced by antigen presenting cells, which synergises with IL-12 to induce IFN-γ [97].

IL-31 is a latterly described cytokine that is mainly produced by activated Th2 cells. It is integrated into T-cell mediated response and is involved in inflammation and degenerative skin diseases. It promotes the induction of IL-6 and IL-8 [100,101,102].

IL -35

IL-35 is a newly discovered cytokine belonging to the IL-12 family. It is generated by the regulatory T cells. Its functional effects are mediated by the release of IL-10. The pivotal role of this cytokine in Treg suppressing function and in decreasing both Th17 cell function and IL-17 release is readily recognized. However, the effect of this cytokine on osteoclasts and osteoblasts warrants further studies [103].

IL -36

IL-36 is a pro-inflammatory cytokine that manifests itself in 3 different forms: IL-36α, β and γ. These molecules have the same biological effects, and the molecular weight of each one is 18kD. The main target of action is on dendritic cells. It helps in their proliferation and maturation. After exposure to IL-36, dendritic cells release IL-12. IL-36 can induce the release of pro-inflammatory cytokines, such as IL-6 or IL-8 as well as IL-17, IL-22, and IL-23 [97,104].

IL -37, IL -38

A major modulator of inflammatory response *in vitro*, IL-37, inhibits the release of pro-inflammatory cytokines, most notably IL-1 family members and TNF. During stressful situations, cytokine directly prevents the release of pro-inflammatory cytokine such as TNF, IL-1β, IL-6 and IL-17.It has no effect on Th2 cytokines [104].

IL-38 is a novel cytokine whose effect may resemble that of IL-36α as it binds to the IL-36 receptor and inhibits its effects, particularly the Th17 response. It decreases the production of IL-17 and increases the production of IL-6 by dendritic cells [104]. Still, the exact role for these cytokines awaits further clarification.

The list of cytokines in which the mechanisms on novel signalling pathway remains unidentified are given below in table 1;

CONCLUSION AND FUTURE PERSPECTIVES

Most of the recent studies discussed above elaborated the role of the majority of cytokines in RANKL/RANK–OPG mechanism. They have concluded beyond doubt, that Osteoimmunology is a discipline that highlights the dynamic interaction between skeletal system and immune cells. Although a large number of researches have been conducted till date on the intrinsic process of this novel pathway, further studies are required for identifying the roles of newer generation of cytokines in the RANKL/RANK mechanism. The discovery of the intricacies of such a mechanism of the unidentified cytokines on this novel pathway will be crucial for treating bone related pathological infirmities in future.

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Table 1: Cytokines in which RANKL/RANK-OPG mechanism is unidentified

Cytokines	Source	Functions	Reference
IL -2	CD4+Th1cells, CD8+ Th1 cells	a)A growth factor for activated T – cells b) It combines with IL-12 to increase cytotoxicity by NK cells	Malek TR. 2008[79]
IL-5	CD4+ Th2 cell	a) B-cell activation, growth b) Eosinophil differentiation factor.	Milburn.MVetal.1993[81]
IL-9	T–lymphocytes	a) Mast cell growth factor b) Major inducer of IL-6 production. c) Promotes the growth of CD4+T cells and Th17 cells.	Hultner L et al.1990[82]
IL-14	T-cells	Growth of B-cells	Shen L et al.2006[83]
IL-16	T cells	Selective migration of CD4 T cells	De Bie JJ et al. 2002[85]
IL-19	LPS activated monocytes.	Modulates Th1 activity	Kotenko.SV.etal.2006[88]
IL-20	Monocytes and keratinocytes	Inflammatory responses of skin	Blumberg et al.2001[89]
IL-22	ActivatedTH1 cells	It induces acute phase proteins and inhibits IL-4 production by Th2 cells	Cella M et al.2009[91]
IL-24	Monocyte, T- cells	It induces cytokines such as IL-1, IL-6 and TNF	Wang M et al.2002[93]
IL-25	Th2 cells, mast cells,macrophages	It induces the production of IL-4, IL-5 and IL-13.	Fallon PG et al.2006[95]
IL-26	T-cell, NK cells.	It increases the production of IL-8 and IL-10	Pene J et al.2008[96]
IL-28	Dendritic cells and monocytes	It impedes viral replication and type 1 IFN like activity	Uzé G et al.2007[99]
IL-29	Dendritic cells and monocytes	a) Stimulates monocytes and macrophages b)Increases the production of IL-6, -8, and -10 c)Inhibits the production of IL-13 by T cells	Meager A et al.2005[98]
IL-30	Antigen presenting cells	It synergises with IL-12 to induce IFN-γ.	Akdis M et al. 2011[97]
IL-31	ActivatedTH2 cells	a) Integral in T-cell mediated response b) In inflammation and degenerative skin disease.	Diveu C et al.2004[100]
IL-35	Regulatory T cells	a) Treg suppressor function b) To decrease both Th17 cell function and IL-17 release	Niedbala W, Wei X-Q, Cai B, et al.2007[103]
IL-36	Dendritic cells	It induces the release of pro-inflammatory cytokines, such as IL-6 or IL-8 as well as IL-17, IL-22, and IL-23.	Gaëlle Clavel G et al. 2013[104]
IL-37	Monocytes, plasma cells	It directly inhibits the release of pro-inflammatory cytokine such as TNF, IL-1β, IL-6 and IL-17.It has no effect on Th2 cytokines.	Gaëlle Clavel et al.2013 [104]
IL-38	B cells	It binds to the IL-36 receptor and deters its effects	Gaëlle Clavel et al.2013 [104]

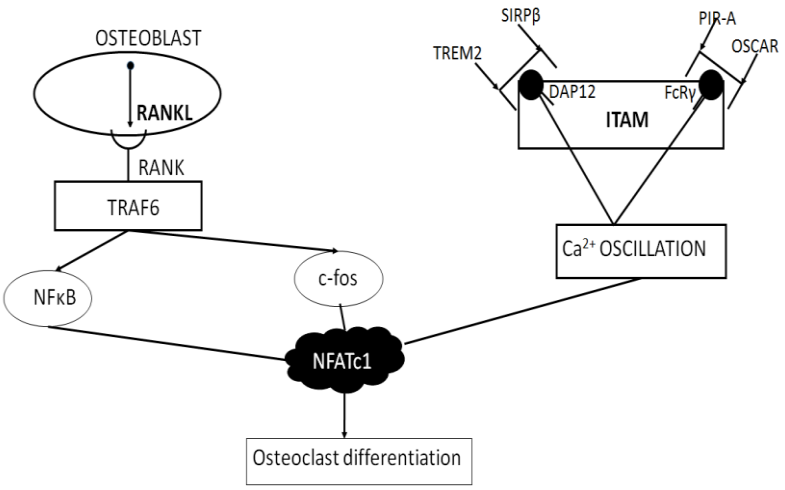

Figure 1: Main pathways of osteoclast differentiation

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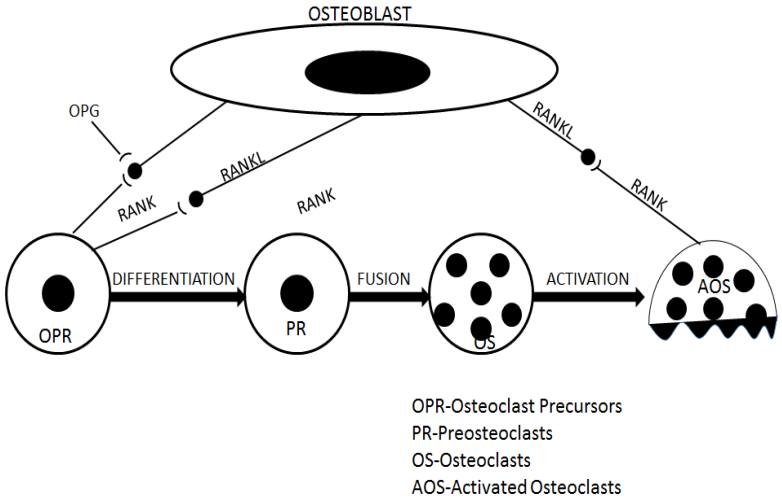

Figure 2: RANKL-RANK-OPG MECHANISM

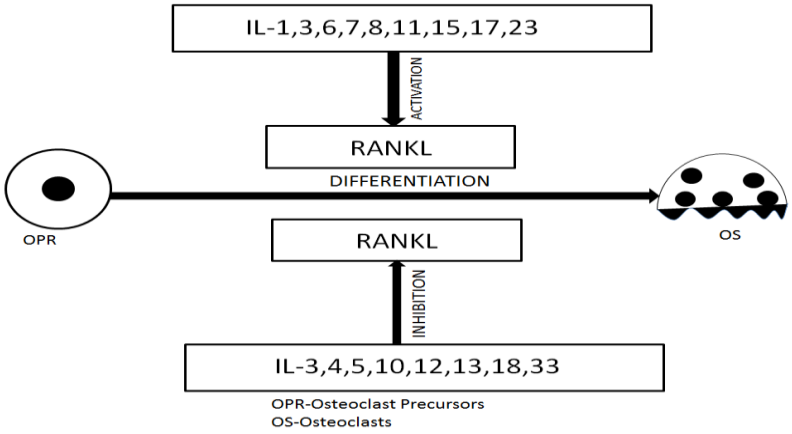

Figure 3: The effect of Interleukins on RANKL mechanism [activation and inhibition]

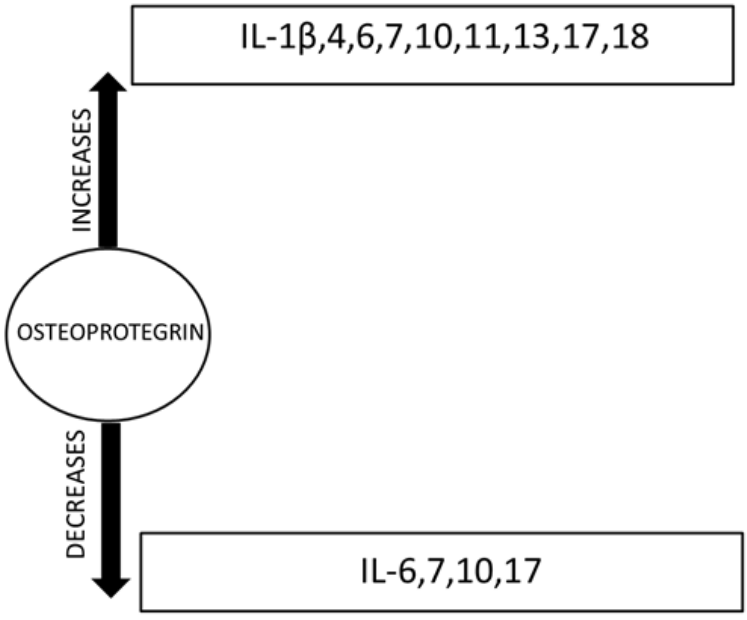

Figure 4: The effect of Interleukins on OPG mechanism [activation and inhibition]

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