The Role of Pro-Inflammatory Mediator Interleukin-32 in Osteoclast Differentiation

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ABSTRACT

The recently explained cytokine, which is produced after the stimulation of interferon (IFN)-γ, interleukin (IL)-2, and IL-18 is IL-32, has pro-inflammatory IFN-γ, IL-2 and IL-18 are IL-32 mediator’s properties that are generally entailed in many diseases, including infections, cancer, and chronic inflammation. After the initial statement in 2005, it promoted the osteoclast precursor’s differentiation into TRAcP plus VNR plus multinucleated cells that express explicit osteoclast indicators. Furthermore, the loss of bone resorption might be accredited because of the collapse of the multinucleated cells, which are produced of the reaction to IL-32 to direct factoring that is ultimately essential for attaching the cells for bone resorption. Thus, in conclusion, IL-32, the pro-inflammatory mediator, has an important and indirect role in regulating osteoclast differentiation. In bone disorder’s pathophysiology, critical role of IL-32 needs more scientific evidence to develop a rational treatment protocol. IL-32 can become a potent mediator of active osteoclast generation in the presence of receptor activator of NF-κB ligand (RANKL). This novel cytokine can introduce more favorable conditions for osteoclastogenesis in the rheumatic arthritis by increasing the RANKL and osteoprotegerin ratio in fibroblast-like synoviocytes.

Key words: Interleukin-32, cytokines, osteoclast, pro-inflammatory

INTRODUCTION

In 1992, the focused molecule in this review was first reported, where there is a protein which was called NK4, which is extremely articulated in activated-T and NK cells. This protein was rapidly up-regulated after the stimulation by phythaemaglutin in a lectin that is primary for activation of T-cells in human peripheral blood mononuclear cells (PBMCs). In 2005, NK4 was found to be one of the most up-regulated genes using microarray expertise and interleukin (IL)-18 receptive cell unit. After that, two other innovative integrins of IL-32 were established in IL-32 mRNA transcript and IL-32γ, but IL-32β appears superabundant. IL-32 different isoforms are produced by splicing of isoform IL-32γ pre-mRNA. Many reports have explained that IL-32 different transcripts present both in vitro and in vivo. Its remnants that, by which means, IL-32γ mRNA copies are replicated and incomplete body cells process is the same. Keeping in mind the cell stimulation and cell demise, IL-32γ is the utmost leading IL-32 isoform, which explains why IL-32γ explodes into less injurious IL-32 isoforms, such as IL-32β and α. IL-32 isoform differential potency was explained in many reports, however, basis of potency differences between the isoforms remain unknown. In the explanation of this process, the variance between the extent of the integrins from 14.9 kDa (IL-32α) to 26.7 kDa (IL-32γ), so that the isoform’s tertian assembly can be explained.5

Expression and regulation of osteoclast

Inlucunarily, bone resorption is the specific function of multinucleated osteoclasts cells that originate from the hematopoietic lineage (colony forming unit-granulocyte–macrophage; CFU–GM).6 The presence of KB ligand nuclear factor by receptor activator and colony-stimulating factor of macrophages is compulsory for the discrepancy of osteoclasts by circulating hematopoietic predecessors.7 The site triggers
for nuclear factor κB ligand (RANKL) is part of tumor necrosis factor (TNF), which is present on T-cells, osteoblasts, and binds with its receptor, a receptor activator for nuclear factor-κB (RANK), which are articulated on precursors of osteoclast. Activation of different intracellular pathways such as mitogen-activated protein kinase, nuclear factor activated T-cells (NFATc1), Akt, and nuclear factor-κB (NF-κB) pathways has been described as a result of RANK binding with RANKL.

Osteoprotegerin (OPG), which acts like RANKL decoy receptor, causes the stimulation of resorbing activity by osteoclasts and blocks the differentiation of osteoclast-mediated by RANKL. Although RANKL is one of the critical factors for osteoclastogenesis, several pro-inflammatory cytokines such as IL-8, TNF-α, and LIGHT proves the RANKL independent mechanisms.

Multiple cell interaction evolute of rheumatoid arthritis (RA)
Approximately 0.5% adult population is affected by rheumatoid arthritis (RA) worldwide, which is the main reason for disability. RA can be defined as an enduring inflammatory disease, in which advanced joint annihilation occur including articular cartilage damage, which is caused by inflammatory cells that are chondrocytes and activated synovial fibroblasts. The factors that produced in the affected joints and a broad array of cytokines control the arthritis evolution. The anti-inflammatory cytokines i.e. IL-10 and transforming growth factor-beta (TGF-β) are exceeded by pro-inflammatory molecules level, particularly monokines TNF-α and IL-1b. The importance of macrophages and cytokine production in RA is clearly explained by biological therapies that were directing TNF-α, targeting IL-1 and IL-6.

However, these treatments, when given repeatedly, achieve only brief clinical responses. Furthermore, approximately 40% of patients with 50% response reach American College of Rheumatology.

Fibroblast-like synoviocytes (FLS) cultures
In sub confluence (70%), FLS were grown which contained complete medium i.e. 10% fetal calf serum in addition to RPMI 1640, 500 units/mL of penicillin, and 100 μg/mL streptomycin in a culture flask. From 3rd passage, all the experiments were performed using FLS. At this time, there were 0-2% contaminating macrophages, natural killer cells, and lymphocytes.

RNA preparation
To eliminate genomic DNA contamination, DNase I are treated with entire RNA, which is obtained after culturing cells in RLT® RNA extraction buffer (Rneasy, Qiagen kit). By using RNA kit 6000 Lab Chip (Agilent Technologies) and a Bio-analyzer 2100, the unity and clarity of the entire RNA, and cRNA, were analyzed. The ratio of total RNA with 28S/18S >1.7 was only used. Through NanoDrop (Nanodrop Technologies) concentrations of cRNA were calculated.

cRNA production and probe range hybridization
As per the producer’s protocol (GeneChip® Expression Analysis Technical Manual, Rev. 5, Affymetrix Inc., 2004) through the GeneChip Expression 3’ Amplification One-Cycle Target Tagging and Controlling Components, cRNA preparation was carried out with 3 lg of entire RNA, then combine with the human genetic material U133 plus 2.0. Briefly, in an initial-strand cDNA composite reaction using a T7-Oligo(dT) promoter primer, the entire RNA was initially inverse transcribed. Then, the double-stranded cDNA was washed in second-strand cDNA synthesis that is facilitated by RNase H and is active as a prototype in the in vitro transcription reaction (IVT). In the presence of a biotinylated nucleotide analog and T7 RNA polymerase, an IVT reaction was performed. Then, biotinylated cRNA marks were washed up, broken into pieces, and hybridized with GeneChip expression arrays. Then, using Affymetrix Fluidics Station 450 (Affymetrix, Inc.), it was washed and stained and then the reviewed ranges were perused into the Affymetrix GeneChip Scanner 3000.

FLS gene express model
Using GeneChip Human Genome U133A plus 2.0 (Affymetrix, Santa Clara, CA, USA), microarrays evaluated the genetic appearance profiles. Gene expression was evaluated by cultivated FLS obtained of 8 and 9 patients with RA and OA, respectively. For further analysis, outcomes from 241 investigations on behalf of 171 different cytokines and their particular receptors. The selected genes, whose appearance were diverse and approximately 1.6 times among the FLS of two disorders, had a p value of up to 0.05.

Microarray scrutiny
In gene spring, the stated raw details were computed with the GC-RMA File preprocessor. Specific probe data stored in Affymetrix CEL files were used using the GC-RMA algorithm. With Genespring 7.2, raw data processing, data analysis, and normalization were performed. The value of each gene was set to 1 in different conditions and it was ensured using GeneSpring normalization (“per gene: normalize the median”). This means that those genes that do not alter in different conditions have a value of 1 for normalization expression that allow easy detection of distinctive expressed genes visually.

The absence of sRANKL IL-32 inspires the discrepancy of supporter PBMCs into multinucleated TRAcP + and VNR + cells
Now it is thought that M-CSF and RANKL are two crucial aspects that are supplied by osteoclasts, which are vital for the maturation and discrepancy of precursors of osteoclasts. Moreover, the mice defective by M-CSF (op/op) exhibit an osteopetrotic appearance that could be voluntarily converse with time and suggest that there is a substitute osteoclastic trail that exists.

Lacking M-CSF, vascular endothelial growth factor, hepatocyte growth factor, and Flt3 ligand all have revealed support to osteoclast creation. Moreover, the mice demonstrate an osteopetrotic appearance triggered by a whole loss of osteoclast in their bones having a deficiency of either RANKL or its receptor RANK. If there are no osteoclasts detected in the bones of the mice that are flawed in RANKL or RANK, it might not happen due to the total disaster of osteoclastogenesis. RANKL as a significant and endurance aspect for modified osteoclasts.
and in the mice deficient with RANKL or RANK, the observed phenotype can be explained by the idea that differentiation is diminished osteoclast superimposed on the summarized lifecycle. As such, in the existence of a large amount of OPG that is an inhibitor of interactions of RANKL-RANK, it has been reported that a substitute RANKL-independent pathway (e.g., LIGHT, TGF-β, and TNF-α) supports osteoclastogenesis. The ground aspect of the osteoimmunology explained that T-cells, which are activated straight regulate bone resorption and osteoclastogenesis, and T-cell products i.e., IL-17, TWEAK, GM-CSF, and IFN-γ, which can modulate the establishment of osteoclasts. This existing study pursued to determine a part of IL-32, having the representation of pro-inflammatory cytokine and participating in an assortment of inflammatory syndromes by osteoclast activation and differentiation (Figures 1, 2).

**TNF and osteoclast activation**

It has been described that TNF receptor-associated factor-6 (TRAF-6) is imperative for osteoclast stimulation, i.e., lacunar bone resorption and there is a composite part of IFN-γ in osteoclastogenesis. They show that strong reluctance of RANKL-induced activation occurs due to fast degradation of TRAF-6 by IFN-γ. Therefore, we hypothesized that due to TRAF-6 degradation, the IL-32 single in or in combination with soluble RANKL showed inhibitory outcome. However, we found and were surprised that TRAF6 is not destroyed but is overexposed, when treated with IL-32 related to RANKL. Recently, Yao et al. have shown IFN-γ shows a "direct" anti-resorptive outcome by reducing the distinction of osteoclasts. Therefore, by stimulating T-cells IFN-γ can act "indirectly" as a pro-resorptive feature to direct RANKL and TNF-α. In this current study, we use PBMCs as a basis of pioneers of osteoclasts and significantly cells were cleansed completely to abolish non-adherent cells (B & T-cells), it is reasonable that few T-cells might be existing in the culture and donated to osteoclastogenesis. This supposition is also strengthened by indication, which explains that the decrease in size and number of multinucleated cells newly-synthesised due to excessive accumulation of OPG in the IL-32-treated cultures.

**Therapeutic techniques or process**

Osteoclast differentiation was induced by IL-32 is somewhat autonomous of the RANK/RANKL pathway. Although the freeing of pro-inflammatory mediators that were increased by IL-32 have a positive influence on osteoclastogenesis, it had a straight inhibitor consequence in vitro osteoclast instigation and it cannot induce these recently-prepared multinucleated cells activation into bone-resorbing osteoclasts. It is important to notice that IL-32 has a straight influence over further cell types i.e., epithelial cells, natural killer cells, T-cells, and monocytes. Downstream pathways are not fully interpreted that involved in osteoclasts in return to IL-32. NF-κB and JNK trail activation are severely increased by PBMC handling of M-CSF/RANKL or M-CSF/IL-32 compared to cultures that are treated with M-CSF. However, Akt pathway activation appeared more complex. Akt pathways are strongly activated by M-CSF/IL-32 or M-CSF treatments compared with M-CSF/RANKL.

**CONCLUSION**

In conclusion, IL-32, the pro-inflammatory mediator, has an important and indirect role in regulating osteoclast differentiation. In bone disorder’s pathophysiology, critical role of IL-32 needs more scientific evidence to develop a rational

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**Figure 1.** The graphic illustration of downriver trails triggered by receptor activator of NF-κB ligand (RANKL). Inconsistency detected in RANKL signaling trails; increased ERK1/2 activation may lead to the activation of downriver goals, which, in fact, can subsidize the incapacity of cells to expose the F-actin ring.

**Figure 2.** The graphic illustration of downriver trails triggered by IL-32. The inconsistency detected among IL-32; Akt activation by IL-32 may lead to the activation of downriver goals, which, in fact, can subsidize the incapacity of cells to expose F-actin ring and resorb in reaction to IL-32.
treatment protocol. IL-32 can become a potent mediator of active osteoclast generation in the presence of RANKL. This novel cytokine can introduce more favorable conditions for osteoclastogenesis in the rheumatic arthritis by increasing the RANKL and OPG ratio in fibroblast-like synoviocytes.

**Ethics**

**Peer-review:** Externally peer-reviewed.

**Authorship Contributions**

Concept: T.N., N.T., Design: T.N., N.T., Data Collection or Processing: A.I., I.R., Analysis or Interpretation: T.N., N.T., A.I., I.R., Literature Search: T.N., N.T., A.I., I.R. F.A.S., Writing: T.N., N.T., A.I., I.R., P.A.S.

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