IL-32 ISOFORMS POTENTIALLY IMPACT BONE AND CARDIOVASCULAR DISEASES IN HIV INFECTION

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BACKGROUND

We have recently demonstrated that the multi-isoform proinflammatory cytokine IL-32 is upregulated by HIV infection and is associated with subclinical CVD in people living with HIV (PLWH). However, mechanisms by which IL-32 isoforms contribute to the pathogenesis of CVD and potentially other chronic diseases like osteoporosis remain unclear. We investigated the impact of IL-32 isoforms α, β and γ on the differentiation of human mesenchymal stem cells (hMSC) and primary human monocytes into osteoblasts or osteoclasts (cells involved in calcium deposition and bone formation or bone resorption, respectively). These cells are involved in processes that are known to be dysregulated in PLWH in the bone (increased osteoporosis) and in the heart (increased non-calcified, vulnerable atherosclerotic plaque). We hypothesize that alterations in IL-32 isoforms seen in PLWH may be a common cause for bone and heart disease seen in that population.

METHODS

Human CD14+CD16- monocytes or hMSC (osteoblasts precursors) were stimulated with recombinant IL-32 isoforms α, β or γ at 500 ng/ml with or without the osteoclast differentiation factor RANKL (30ng/ml) for 21 days. Immunofluorescence imaging on Spinning-Disc Zeiss AxioObserver was used to identify and analyze the differentiated cells.

RESULTS

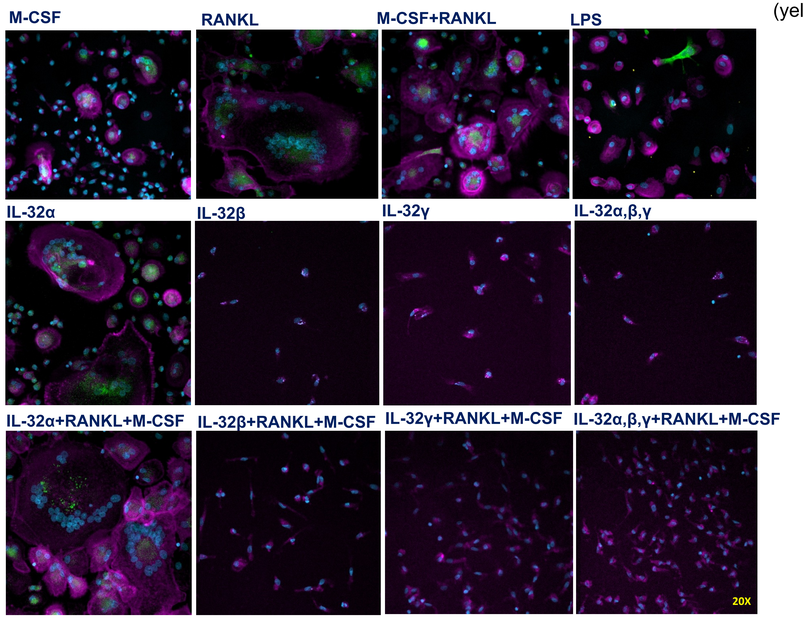


Figure 1. Representative immunofluorescence imaging on Spinning-Disc Zeiss AxioObserver (20X/08) showing the impact of IL-32 isoforms compared to the osteoclastogenic molecules RANKL and M-CSF on monocytes differentiation into osteoclasts. Osteoclasts are defined as cells with the expression of TRAcP5 (green), F-Actin ring (magenta), and multiple nuclei in blue (≥3, identified by DAPI staining). LPS was used as a control.

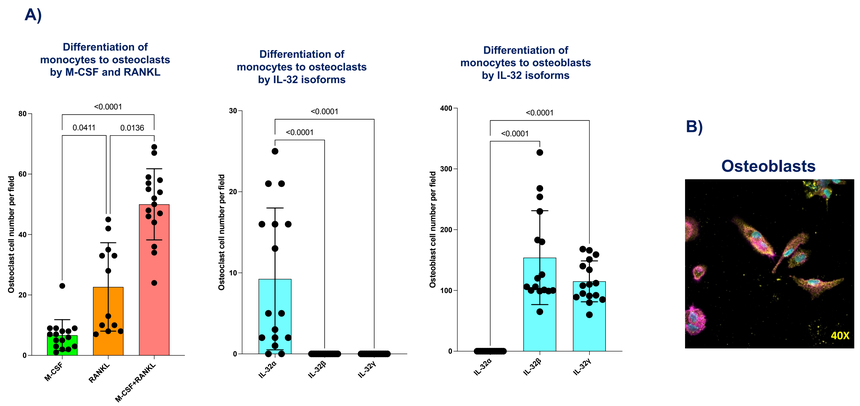


Figure 2. Impact of IL-32 isoforms on osteoclast and osteoblast differentiation from monocytes. A) Left panel: monocyte-derived osteoclast differentiation by osteoclastogenesis-specific mediators RANKL and M-CSF. Isolated CD14+CD16- classical monocytes were stimulated with M-CSF (25ng/ml), and/or RANKL (30 ng/ml) for 21 days (1million cells/well cultured on sterile glass cover slip in 24 well plate). Half media changes were carried out every 3rd day with the supplement of respective stimulant. Osteoclasts were counted manually based on the 3 typical phenotypes (giant multi-nucleated cells with the expression of F-actin ring and TRAcP5) using Zeiss Zen 3.2 software. Middle panel: Number of osteoclast cells differentiated from monocytes treated with IL-32α, β, or γ (500ng/ml) as in the left panel. Right panel: Number of monocyte-derived osteoblasts under co-stimulation with IL-32 isoforms as in the middle panel. Shown is the number of cells counted per field on 20X magnification from n=4 independent donor's cells. Osteoblasts are defined as single-nucleated cells expressing the osteocalcin (yellow) as in **B**. Nonparametric Kruskal-Wallis test and Dunn's subtests were performed for statistical analysis.

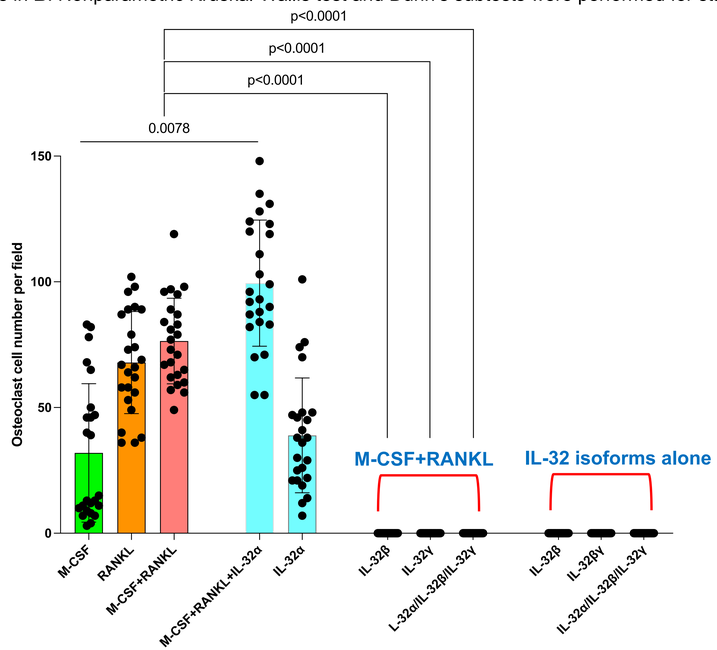


Figure 3. IL-32 β and IL-32γ block the osteoclastogenesis potential of RANKL and M-CSF. While IL-32α enhances the differentiation of M-CSF/RANKL treated monocytes into osteoclasts, IL-32**β** and IL-32**γ** block the osteoclastogenesis effect of M-CSF and RANKL. Data shown are calculated from per condition from n=6 donors. Nonparametric Kruskal-Wallis test and Dunn's subtests were performed for statistical analysis.

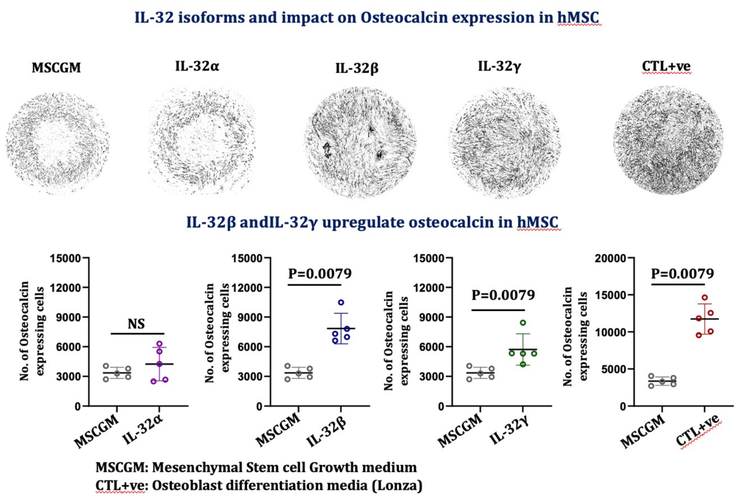


Figure 4. Impact of individual IL-32 isoforms on osteoblast differentiation from human mesenchymal stem cells (hMSC)

hMSC were cultured and maintained as per instructions from the manufacturer (Lonza, PT-2501). Cells were stimulated individually with IL-32 α, β, or γ (500ng/ml) and cultured on 96 clear-bottom well plate for 3 weeks along with negative and positive control condition (growth medium or osteoblast differentiation medium, respectively). Half media changes were carried out every 4th day with supplement of respective stimulants. Upper panels: representative images for osteocalcin expression per condition (Dark area represent osteocalcin-expressing cells). Lower panels: Differentiated osteoblasts in individual IL-32 isoform-stimulated hMSC and positive control-stimulated cells compared to negative control (N=5 experimental replicates). Osteocalcin+ mononucleated cells were counted using FIJI ImageJ software on each images of complete well. Nonparametric Mann-Whitney was performed for statistical analysis. MSCGM: Mesenchymal Stem cell Growth medium used as negative control (Lonza, PT-3001)., Positive Control: Osteogenic hMSC differentiation media (Lonza, PT-3002)

CONCLUSIONS

Our data reveal a new function for IL-32 isoforms β and γ (the longest and dominant IL-32 isoforms) in inducing the differentiation of primary monocytes into osteocalcin+ osteoblasts by counteracting the osteoclast activation factor RANKL. Meanwhile, IL-32α induces the differentiation of osteoclasts. This novel demonstration of the role of IL-32 on the differentiation of cells regulating calcium metabolism suggests that it may constitute a common, upstream cause to patterns of bone and heart disease observed in PLWH. Work is in progress to study the dominantly-expressed IL-32 isoforms in individuals with both diseases.