Regulation of Bone Resorption by Isoforms of Osteoclast Associated Immunoglobulin-Like Receptor

Erin Talbot

College of Dentistry, University of Florida

Bone resorption is controlled by cells known as osteoclasts, whose growth is controlled by the osteoclast associated immunoglobulin-like receptor (OSCAR) gene. The aim of this study was to identify the effects of proinflammatory cytokines TNF-α, IL-1, and LPS on OSCAR-s and OSCAR-m expression in HUVEC and THP cells. Cell cultures were plated, stimulated by a proinflammatory cytokine, and lysed. The RNA collected was then purified, reverse transcribed, and quantitated using real-time PCR. After 24-hour stimulation periods, OSCAR-m was consistently higher in THP cells treated with IL-1 and LPS, but not in cells treated by TNF- α. OSCAR-s control copy numbers were elevated in THP cells treated by all three cytokines. HUVEC cells treated by the three cytokines had increased quantities of both OSCAR-s and OSCAR-m. This suggests that the two isoforms of OSCAR may have different biologic functions.

INTRODUCTION

Osteoclasts are cells that destroy bone and are active in people who suffer from rheumatoid arthritis and other chronic inflammatory disorders (1,2). OSCAR regulates monocyte differentiation into osteoclasts. Osteoclast associated immunoglobulin-like receptor (OSCAR) gene has two groups of isoforms: soluble OSCAR (OSCAR-s) and membrane bound OSCAR (OSCAR-m) (3). The expression of OSCAR gene in monocytes is necessary for their differentiation into osteoclasts, which cause bone resorption (3). Upon ligand binding, OSCAR is activated in monocytes and neutrophils, leading to complex signaling cascades (4). Proinflammatory cytokines, such as interleukin (IL)-1, tumor necrosis factor (TNF)-α, and lipopolysaccharide (LPS), are known to induce bone resorption (4). Here, we examined the expression of soluble and membrane-bound isoforms of OSCAR by monocytes and endothelial cells in response to simulation.

METHODS

Human umbilical vein endothelial cells (HUVEC) were plated and grown in vitro. THP-1, a human monocytic leukemia cell line, was also maintained in culture. The cells were split and re-plated the day before the experiment. Cells in three plates were stimulated for 24 hours by TNF-α, IL-1, or LPS, and the fourth unstimulated dish served as a control group. When studying the effect of cytokines on the presence of OSCAR in THP cells, three concentrations of each cytokine were tested. The cytokine concentrations were controlled at 1μg/ml when studying the HUVEC cells.

In both cases, the cells were lysed, and total RNA was purified using Qiagen's RNeasy assay kit. The RNA was then reverse transcribed to cDNA with Invitrogen's SuperScript First-Strand Synthesis kit. The expression of OSCAR-s and OSCAR-m were then tested by using the cDNA to perform real-time PCR. The primers used for PCR were specific for OSCAR-s and OSCAR-m, and a GAPDH primer was used as a control primer.

The lowest copy number of the tested concentrations run with GAPDH was set as the standard 1:1 ratio. The remaining tested concentrations were compared with the 1:1 ratio to obtain ratio values for those as well. These ratios were then multiplied by the original copy numbers of the OSCAR-s and OSCAR-m data to obtain the correct copy number. The correct copy number from all three trials was averaged and compared to the control value to obtain the % Change Compare to Control.

RESULTS

When THP-1 cells were stimulated by 0.1 ng/ml of TNF-α, the expression of OSCAR-m had very minimal change. The three trials resulted in -26.81%, 0.56%, and 28.24% change in copy number compared to the control copy number values, which averaged to 0.66%. However, the cells stimulated by 1 ng/ml had an average increase of 137%, derived from percentages 211.94%, -78.36%, and 279.46%. When stimulated by 10 ng/ml TNF-α, there was a -19.98%, -24.65%, and 32.35% change in OSCAR-m expression compared to the control copy numbers, averaging a 4% decrease in OSCAR-m expression. As seen in Figure 1, no obvious trend can be identified in the change in expression of OSCAR-m in THP-1 cells after 24 hours of stimulation by TNF-α.

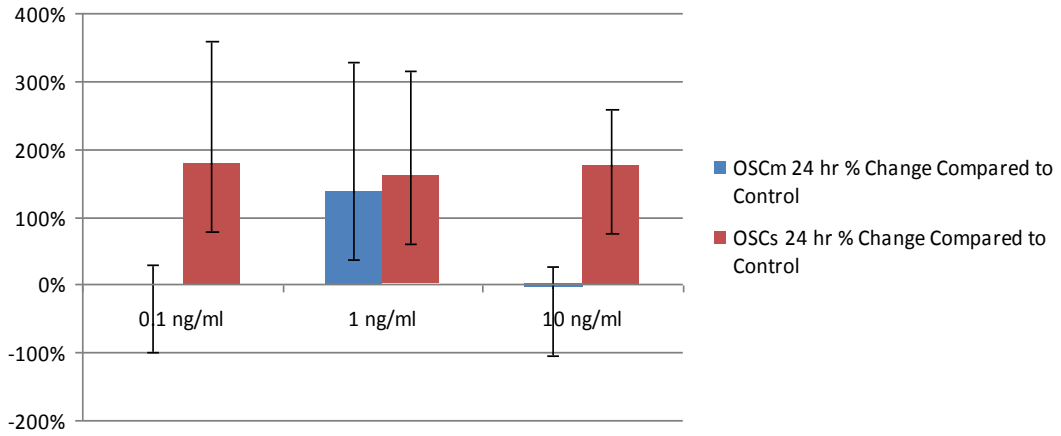

Figure 1: 24 hours TNF-Alpha compared to control

Figure 1 shows that OSCAR-s increased consistently in all three concentrations. The three samples stimulated by 0.1ng/ml of TNF-α had concentrations 215.89%, 337.38%, and -18.01% of the control copy number, resulting in an average of 178.42%. When stimulated by 1 ng/ml of TNF-α, the expression of OSCAR-s had an average increase of 160.36%, with three trial values of 99.82%, 46.08%, and 335.18% in comparison to their control copy numbers. The third group of samples was stimulated by 10 ng/ml of TNF-α and increased OSCAR-s expression by 79.43%, 219.09%, and 228.21%, averaging 175.58%. The average expression of the three concentrations of cytokine stimulation were close in value, showing that TNF-α consistently increased the expression of OSCAR-s in THP-1 cells after 24 hours of stimulation. However, among the three trials for each given cytokine concentration, the percentage change in copy numbers varied.

When testing the effects of IL-1 stimulation on THP-1 cells, both OSCAR-m and OSCAR-s expression was increased, as shown in Figure 2. There was an average increase in OSCAR-m expression of 55.01% compared to the control value when THP-1 cell samples were stimulated with 10 pg/ml over a period of 24 hours. This was derived by individual trial values of -26.08%, 289.30%, and -98.18%. The THP-1 cells stimulated by 100 pg/ml resulted in expression 144.99%, -56.49%, and 477.64% of the control copy numbers, averaging a 98.76% increase in OSCAR-m expression. Cells stimulated by 1000 pg/ml of IL-1 had OSCAR-m copy numbers -20.17%, -72.20%, and 477.64% of the control copy numbers, averaging a 128.42% increase in OSCAR-m expression. The lowest percentage change was seen in 10 pg/ml and the highest percentage increase was seen in 1000 pg/ml treatment, showing that an increase in concentration of IL-1 used to stimulate THP-1 cells over a 24-hour period will also increase the degree to which OSCAR-m expression is increased.

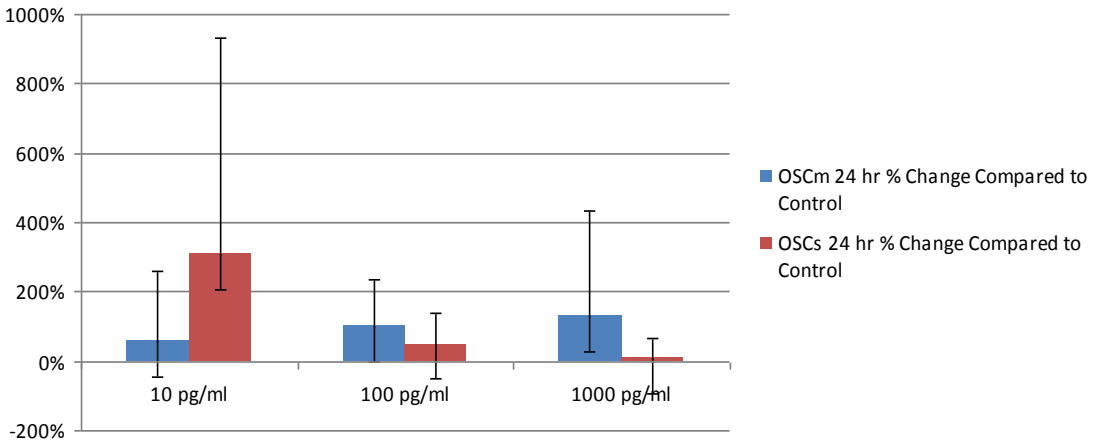

Figure 2: 24 hours IL-1 compared to control

Stimulation of THP-1 cells for 24 hours by 10 pg/ml of IL-1 had a -84.43%, -19.79%, and 1029.69% change in OSCAR-s expression when compared to the control copy numbers, averaging an increase of 308.49%. Treatment with 100 pg/ml of IL-1 caused 135.43%, 51.95%, and -42.96% changes in copy number, averaging a 48.14% increase in OSCAR-s expression. The third group of THP-1 cell samples stimulated for 24 hours by 1000 pg/ml IL-1 increased OSCAR-s expression by 60.71%, 21.38%, and -57.43%, averaging a 8.22% increase. Although treatment with all three concentrations of cytokines increased, the expression of OSCAR-m percentages differs greatly. Figure 2 shows that stimulation by 10 pg/ml of IL-1 has a greater impact on increasing OSCAR-m expression after 24 hours than stimulation by 100 pg/ml or 1000 pg/ml of IL-1.

THP-1 cells were then stimulated for 24 hours with LPS. When treated with 1 ng/ml of LPS, there was 275.46%, 8855.77%, and 5712.19% increases in OSCAR-m expression, averaging 4947.81% more OSCAR-m than the control copy number. Stimulation with 10 ng/ml for 24 hours led to 131.46%, 5366.76%, and 1577.75% increases, averaging a OSCAR-m copy number 2358.66% higher than the control copy number. The greatest increase in OSCAR-m was observed when THP-1 cells were stimulated with 100 ng/ml of LPS. The copy number of OSCAR-m increased 5915.77%, 48138.04%, and 55309.80%, averaging a 36454.54% increase in OSCAR-m expression. This data in Figure 3 shows that OSCAR-m expression in THP-1 cells is much more sensitive to treatment by LPS for 24 hours than to treatment with TNF-α or IL-1.

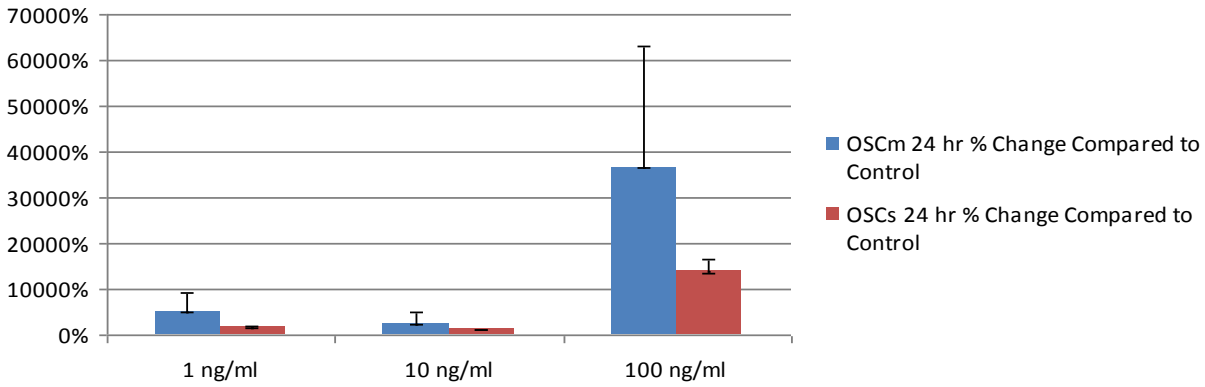

Figure 3: 24 hours LPS compared to control

Stimulation of THP-1 cells for 24 hours with LPS increased the expression of OSCAR-s as well. When treated with 1 ng/ml of LPS, the expression of OSCAR-s was 1196.12%, 1474.06%, and 2010.10% higher than the control copy number, averaging a 1560.09% increase. Treatment with 10 ng/ml of LPS resulted in 983.91%, 1181.20%, and 1151.07% increases in OSCAR-s expression, averaging a 1105.39% increase in copy number when compared to the control copy number. The greatest change in OSCAR-s expression was observed when THP-1 cells were stimulated with 100 ng/ml of LPS. This led to 16359.58%, 13690.94%, and 11006.06% increases in OSCAR-s copy number, averaging an increase of 13685.53%. This data showed that THP-1 cells are more sensitive to treatment by LPS for 24 hours than they are to treatment with TNF-α or IL-1 when measuring OSCAR-s copy number.

The same tests were run with HUVEC in 24-hour periods. These results are displayed in Figure 4. When treated with 1 µg/ml of TNF, OSCAR-m expression increased 294.01%, 37.29%, and 78.42%, averaging 136.57%. Stimulation with 1 µg/ml IL-1 resulted in 35.93%, -38.61%, and 2128.41%, averaging 708.58% increase in OSCAR-m expression. The third group of HUVEC stimulated by 1 µg/ml LPS resulted in -50.98%, -11.64%, and -88.24% changes in OSCAR-m expression, averaging -50.29%. Results show that HUVEC response to IL-1 increased the expression of OSCAR-m the most in comparison to stimulation by TNF-α or LPS.

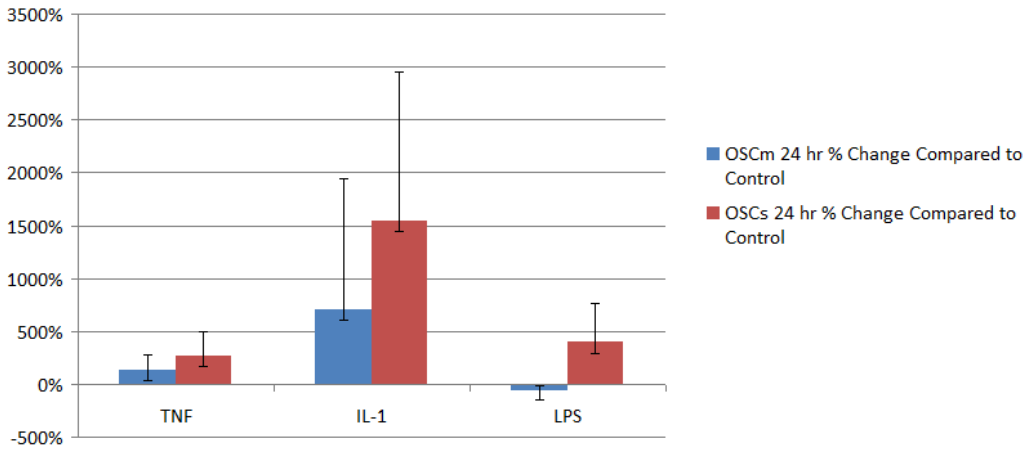

Figure 4: 24 hours HUVEC compared to control

The last group tested the effects of the three cytokines on HUVEC OSCAR-s expression. Stimulation with 1 μg/ml of TNF for 24 hours caused a 65.59%, 524.88%, and 213.29% increase compared to the control copy number, averaging 267.92%. The second set of samples was stimulated by 1 μg/ml of IL-1 and had a 31.12%, 1762.06%, and 2836.48% increase in OSCAR-s expression, averaging 1543.22%. The HUVEC stimulated by 1 μg/ml LPS showed a change of 22.90%, 770.78%, and 395.02% in OSCAR-s expression compared to the control copy numbers, averaging a change of 396.23%. The same trend holds true for OSCAR-s expression in HUVEC as OSCAR-m. Of the three cytokines, IL-1 had the greatest impact on OSCAR-s expression after 24 hours of stimulation.

CONCLUSION

Within the limits of this preliminary study, it can be concluded that THP-1 and HUVEC cells show differential expression of soluble and membrane bound isoforms of OSCAR in response to stimulation by proinflammatory cytokines. This also leads us to believe that OSCAR-s and OSCAR-m may thus have different biological functions. Given the large standard deviation, we will be repeating these experiments to establish reproducibility. Further studies may give insight as to the effect TNF-α, IL-1, and LPS have on the two isoforms of OSCAR. Those findings would be useful in developing treatment for cases in which excess bone resorption causes disease, such as rheumatoid arthritis or periodontal disease.

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