A Paradoxical Effect of Interleukin-1 on the Osteogenic Differentiation of Human Mesenchymal Stem Cells

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INTRODUCTION
Bone marrow contains a population of mesenchymal stem cells (MSCs) with the ability to differentiate into osteoblasts. The osteogenic differentiation of MSCs is central to the regeneration of bone, whether during natural fracture healing or the repair of osseous lesions by tissue engineering approaches. Under many circumstances osteogenesis will occur in an inflammatory environment, such as produced by trauma, infection or surgery, for instance. Whereas many studies have investigated the effect of potent bone inducers such as the Bone Morphogenetic Proteins (BMPs), the effect of inflammatory mediators on osteogenic differentiation has not been well studied and there are few prior data using human osteoprogenitor cells. To address this issue, we have performed in vitro studies to investigate the influence of interleukin-1β (IL-1β), an important pyrogenic cytokine, on the osteoblastic differentiation of MSCs derived from human bone marrow.

METHODS
Human MSC (hMSC) osteoblastic differentiation
In accordance with an IRB-approved protocol, bone marrow-derived MSCs were isolated from the intramedullary canal of patients undergoing hip hemiarthroplasty, and cultured as previously described. Cells (from the second passage) seeded into 24-well plates (5000 cells/cm²) were cultured with medium containing 50 μg/mL ascorbic acid-2-phosphate and 10 nM β-glycerophosphate, supplemented with 100 nM dexamethasone (dex), 10 ng/ml recombinant human IL-1β or 100-500 ng/ml recombinant human Bone Morphogenetic Protein-2 (rhBMP-2). Cells were maintained under these treatment conditions for 21 days, sufficient for MSC osteoblastic differentiation to occur.

Evaluation of MSC osteoblastic differentiation
Osteoblastic differentiation was assessed by the induction of alkaline phosphatase (ALP) enzyme activity, the deposition of a mineralized matrix, and the induction of several transcripts associated with the process of osteogenesis. The nature of the mineral deposited by the cells was evaluated by Fourier transform infrared microscopy (IR)².

Evaluation of signaling pathways stimulated in MSCs by IL-1β
Activation of mitogen-activated protein kinase (MAPK), Phosphoinositide 3-kinase (PI-3K)/Akt and mothers against Decapentaplegic Drosophila (SMAD) pathways by IL-1β was assessed by immunoblotting. The possible role of MAPK, PI-3K and SMAD pathways in IL-1β-mediated hMSC osteoblastic differentiation was evaluated by blocking these pathways using specific inhibitors.

RESULTS
Effect of IL-1β on hMSC osteoblastic differentiation
Unexpectedly, IL-1β-treated hMSCs showed matrix mineralization to the same extent as dex treated cells (Figure 1a). IR analysis of the mineral indicated that both IL-1β and dex treated MSCs formed crystalline hydroxyapatite characteristic of bone mineral (Figure 2). In agreement with previous studies, rhBMP-2 did not lead to calcium deposition by hMSCs (Figure 1b).

IL-1β failed to induce any transcript associated with osteoblastic differentiation. In addition, IL-1β was unable to induce alkaline phosphatase activity (Figure 3), an enzyme considered necessary for the liberation of the phosphate required for mineralization.

DISCUSSION
These results suggested that IL-1β has the paradoxical effect of promoting the deposition of authentic bone mineral by hMSCs without inducing the expression of established osteoblast markers, whereas BMP-2 induces the expression of osteoblast transcripts without enabling mineral deposition. The molecular mechanisms through which IL-1β promotes mineralization without inducing ALP need to be elucidated. This paradoxical effect of IL-1β may explain why extensive or ectopic bone formation is observed in inflammatory conditions such as osteomyelitis and osteitis and provide new insights for improving the success of MSC-based bone healing strategies.

REFERENCES