Pro-inflammatory Mediators Stimulate the Osteogenic Commitment of Human Bone Precursor Cells

Michiel Croes, MSc¹, Cumhur C. Oner, PhD, MD¹, Moyo C. Kruyt, PhD, MD¹, Wouter JA Dhert, PhD, MD¹,², Jacqueline Alblas, PhD¹. ¹University Medical Center Utrecht, Utrecht, Netherlands, ²Faculty of Veterinary Medicine, Utrecht University, Netherlands.

Disclosures:  

Introduction:  
Although uncontrolled inflammation has destructive effects on bone, local inflammatory processes also underly new bone formation during fracture repair or in heterotopic ossifications [1, 2, 3]. Our aim is to identify inflammatory mediators with pro-osteogenic effects on multipotent stromal cells (MSCs) and the specific conditions allowing these effects. This can be implemented as a component of bone replacement strategies alternative to the autologous bone graft. Here, we studied the effects of the pro-inflammatory cytokine tumor necrosis factor-alpha (TNF-α) and the bacterial component lipopolysaccharide (LPS) on the osteogenic differentiation on human MSCs. To determine in which stage of lineage commitment pro-inflammatory mediators exert their effect on osteogenic differentiation, the role of exposure time and cell maturation stage was studied. Results were compared for the differentially acting osteogenic inducers BMP-2 and dexamethasone.

Methods:  
Human bone marrow-derived MSCs were cultured in normal growth medium (control) or osteogenic differentiation medium (rhBMP-2 or dexamethasone/L-ascorbic acid 2-phosphate). When matrix mineralization was studied, β-glycerophosphate was added in the culture medium. MSCs were treated with different doses of rhTNF-α or LPS using experimental designs. To discriminate between early and late osteogenic commitment, MSCs were pre-differentiated for 12 days prior to start of the experiment. To study short exposure effects, pro-inflammatory mediators were withdrawn from the culture after 48 h. Alkaline phosphatase (ALP) activity was determined by conversion of the p-nitrophenyl phosphate Liquid Substrate System and normalized to DNA content (Quant-It PicoGreen kit). This was confirmed by staining with the Fuchsin and Chromogen-Substrate system. As a late marker of osteogenic differentiation, binding of the calcium-chelating fluorochrome xyleneol was quantified. Using the ArrayScan XTI fluorescence in combination with Cellomic VHS software, the percentage of calcium-positive and negative cells was determined. Matrix mineralization was also assessed by Alizarin Red S staining. An immunohistochemical staining for osteocalcin was performed as a third marker of osteogenic differentiation. Results were represented as the mean ± standard deviation for two MSC donors. The values for individual donors were based on triplicate measurements. Differences between the groups were considered significant compared to the control when p<0.05, calculated by two-tailed Student’s t-test.

Results:  
Following osteogenic induction by dexamethasone or BMP-2, TNF-α and LPS synergistically enhanced the ALP expression of MSCs in a dose-dependent manner (Fig. A, B). Compared to the groups with dexamethasone or BMP-2 alone, 5 ng/ml TNF-α concentration induced an approximately 4-fold and a 1.5-fold increase in ALP expression, respectively. Treatment with LPS resulted in an almost 3-fold increase in BMP-2 or dexamethasone induced ALP activity. Consistent with the early changes in ALP expression, staining for calcium at 22 days demonstrated increased matrix mineralization for treatment with an inflammatory mediator after osteogenic induction (Fig. C). Short TNF-α/LPS treatment only affected the ALP expression in dexamethasone differentiating cells where the optimal TNF-α and LPS concentration resulted in 2.5 to 3-fold increases in ALP levels compared to the untreated group. TNF-α or LPS treatment of pre-osteoblasts for 14 days always stimulated their matrix mineralization and the relative responses to TNF-α and LPS were largest in dexamethasone and BMP-2 pre-differentiated cells, respectively. Treatment of dexamethasone pre-differentiated cells with 50 ng/ml TNF-α for 14 days resulted in 10-times more calcium positive cells compared to cells treated with dexamethasone alone. Using 0.5 μg/ml LPS treatment, a 6-fold increase in calcium depositing cells was found following BMP-2 pre-differentiation.

Discussion:  
The very potent inflammatory mediators TNF-α and LPS stimulate the osteogenic commitment of human bone precursor cells, whereby synergistic effects occur in combination with well known osteogenic inducers. As such, results were similar following dexamethasone or BMP-2 induction, for which related effects are mediated by different signaling pathways. This suggests that pro-inflammatory signals interfere with common downstream regulators of osteogenic differentiation in bone progenitor cells.  

Significance:  
The co-delivery of BMPs and pro-inflammatory mediators may thus provide a stronger pro-osteogenic signal to bone precursors in bone replacement strategies.

Acknowledgments:  
The authors are grateful to Okan Bastian for his assistance during xyleneol orange analysis. David Egan is acknowledged for his...
excellent assistance in the scanning and analyses of these samples. Hendrik Gremmels is thanked for his help during characterisation of MSCs.

References: