Osteoprogenitor Cells Exposed to Polymethylmethacrylate Particles during the Proliferation Stage Are Susceptible to Irreversible Inhibition of Osteoblastic Differentiation

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Statement of Purpose: Particulate wear debris is implicated as a significant inhibitory factor of osteoblastic differentiation, proliferation, and function. The stage of osteoprogenitor proliferation, the initial phase in the osteoblastic differentiation pathway, may be a crucial time period during which immature osteoprogenitors are most sensitive to inhibition by polymethylmethacrylate (PMMA) particles. This study investigated the time-dependent effects of PMMA particles on osteoprogenitor differentiation during the proliferation stage.

Methods: Bone marrow cells from C57 mice were cultured in osteogenic medium containing 10^{-7} M dexamethasone, 10 mM β-glycerophosphate, and 50 μg/ml ascorbic acid. PMMA particles (1-10 μm, Polysciences) were added to bone marrow cell cultures at a concentration of 0.30% v/v on day 0 of differentiation in osteogenic medium. Cells were monitored for the rise in cell number and alkaline phosphatase expression each day throughout the first 8 days of differentiation. Three separate cultures were maintained with PMMA particles for 1, 3, and 5 days respectively, after which the particles were removed by thorough washing with PBS. These cells were allowed to grow for a total period of 15 days in osteogenic medium, after which the cultures were: (1) measured for the quantity of DNA using a PicoGreen DNA quantitation kit (Molecular Probes), (2) stained for alkaline phosphatase using a staining kit from Vector, and (3) stained for mineralized nodules by the von Kossa method. Positive controls were challenged with particles throughout the entire culture period; negative controls were grown without particles. The quantity of alkaline phosphatase and von Kossa stains in each culture well was measured using NIH Image, which generates a histogram value representing the total stained area in the culture well out of a maximum of 256 units. Statistical analysis of the histogram data and DNA measurements was performed using ANOVA and Fisher's PLSD.

Results / Discussion: Bone marrow cells co-cultured with PMMA particles throughout the entire culture period showed no rise in cell number and alkaline phosphatase expression (Figure 1), demonstrating that uninterrupted particle exposure causes complete suppression of osteoprogenitor differentiation (results for alkaline phosphatase not shown). Cells challenged with particles only during the first 5 days of differentiation yielded approximately the same levels of proliferation, alkaline phosphatase expression, and mineralization as positive controls challenged throughout the entire culture period (Figures 2-4), which indicates that a 5-day particle treatment period was sufficient to cause complete, irreversible inhibition of osteoprogenitor differentiation. Cells challenged with particles for ≤ 3 days yielded levels of proliferation, alkaline phosphatase expression, and mineralization that were significantly less than the negative controls but also greater than the positive controls (Figures 2-4), indicating that particle challenge for less than 5 days resulted only in partial inhibition of osteoprogenitor differentiation. The irreversibility of these inhibitory effects may be due to osteoprogenitor inactivation after phagocytosing particles or to cytotoxic effects of particles on the osteoprogenitors. Studies have shown that the maturational age of osteoblasts determines the responsiveness of these cells to physiologic stimuli. Certain growth factors and hormones, for example, exert their effects primarily on relatively undifferentiated osteoprogenitors in the proliferation stage. Similarly, particles may alter the program of development in immature, proliferating osteoprogenitors.

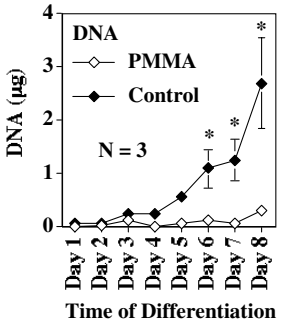

Figure 1.

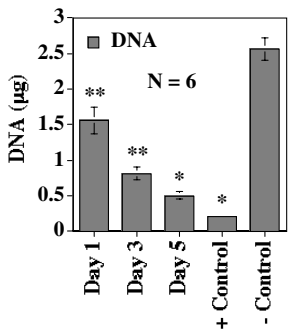

Figure 2.

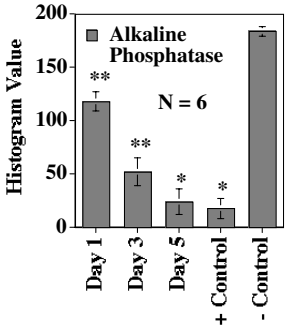

Figure 3.

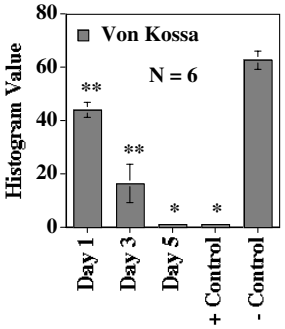

Figure 4.

Figures: Day 1, 3, and 5 on the horizontal axis in Figures 2-4 represent the time when particles were removed from culture. Histogram values represent the total stained area out of a maximum of 256 units. * $p < 0.05$ vs. negative control. ** $p < 0.05$ vs. both positive & negative controls.

Conclusions: This study demonstrates that the stage of osteoprogenitor proliferation is a crucial time period during which differentiating osteoprogenitors are most sensitive to inhibition by PMMA particles in vitro. Osteoprogenitors challenged with PMMA particles during the first 5 days of differentiation show complete, irreversible inhibition of osteoblastic development. Beyond this 5-day period, the inhibitory effects are sustained even after particles are removed from culture.

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References: Goodman SB. Acta Orthop Scand Suppl 1994; 258: 1-43.