Undifferentiated Marrow Stromal Cells Lose Their Osteogenic Capability After Exposure to Polymethylmethacrylate Particles in a Non-Osteogenic Environment

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Statement of Purpose: Periprosthetic bone loss may be a combined effect of particle-induced osteolysis and suppression of osteoblastic differentiation. It is important to determine whether stem cells and osteoprogenitors in the bone marrow function normally under chronic exposure to wear particles. Inhibition of osteoblastic differentiation by wear particles may result in decreased osteoblast production, which will compromise bone regeneration and remodeling in the prosthetic bed. This study investigated the effects of polymethylmethacrylate (PMMA) particles on bone marrow stromal cells in non-osteogenic medium and the subsequent ability of these cells to differentiate into osteoblasts.

Methods: Bone marrow cells from C57 mice were cultured in non-osteogenic medium composed of DMEM with 15% FBS and antibiotics. Cell cultures were challenged with PMMA particles (1-10 μm, Polysciences) at concentrations of 0.038%, 0.075%, 0.150%, 0.300%, and 0.600% v/v in non-osteogenic medium for 15 days, after which the cultures were measured for the quantity of DNA (DNA measurements were taken at 5-day intervals for the cultures challenged with 0.300% v/v PMMA). Separate cultures were challenged with the same doses of PMMA particles in non-osteogenic medium for 5 days, after which the particles were removed by thorough washing with PBS. The cells were then grown for 15 days without particles in osteogenic medium containing 10^{-7} M dexamethasone, 10 mM β-glycerophosphate, and 50 μg/ml ascorbic acid. The cultures were subsequently: (1) measured for 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. The quantity of alkaline phosphatase and von Kossa stains in each culture well was measured using the software program 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 cell cultures challenged with PMMA particles in non-osteogenic medium for 15 days showed a 4.5-, 5.3-, and 5.4-fold increase in cell number at particle doses 0.038%, 0.075%, and 0.150% v/v respectively, but there were no significant changes in cell number at particle doses 0.300% and 0.600% v/v (Figure 1). Cells challenged with 0.300% v/v PMMA showed approximately the same cell number as the negative control throughout the 15-day culture period (results not shown). Cells grown without particles in osteogenic medium for 15 days after being incubated with particles in non-osteogenic medium for 5 days showed a dose-dependent decrease in cell proliferation, alkaline phosphatase expression, and mineralization, with a sharp decline at particle dose 0.300% v/v (Figures 2-4). These results indicate that marrow stromal cells lose their potential to differentiate into osteoblasts after being pre-treated with particles in non-osteogenic medium. This permanent loss in osteogenic potential may be due to: (1) the ability of stem cells and osteoprogenitors in non-osteogenic medium to enter an inactivated state after phagocytosing particles, (2) cytotoxic effects of particles on the viability of marrow stromal cells, or (3) the release of inhibitory factors by other particle-activated cell types in the adherent cell culture. The huge proliferation spike observed with cells challenged with particles in the 0.038% - 0.150% v/v dose range in non-osteogenic medium (Figure 1) could be due to particles' ability to stimulate non-stem-cell, non-osteoprogenitor populations in the heterogeneous culture to proliferate.

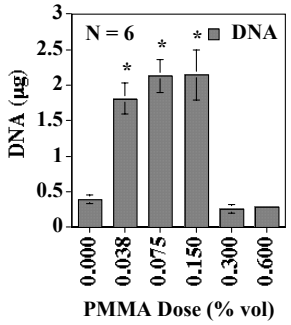

Figure 1.

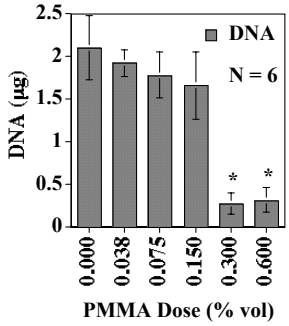

Figure 2.

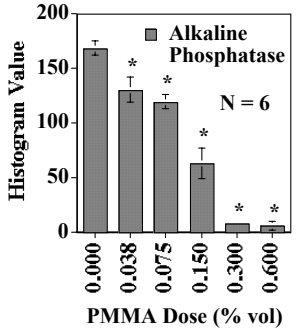

Figure 3.

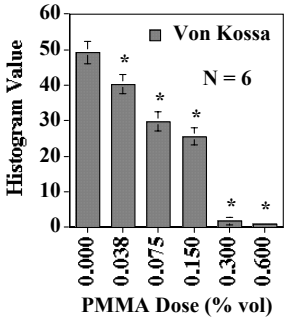

Figure 4.

Figures: Cells in Figures 2-4 were incubated with particles in non-osteogenic medium for 5 days and then grown without particles in osteogenic medium for 15 days. Histogram values represent the total stained area out of a maximum of 256 units. * $p < 0.05$ vs. control.

Conclusions: This study demonstrates that PMMA particles deprive bone marrow stem cells and osteoprogenitors in non-osteogenic medium of their ability to subsequently differentiate into osteoblasts after exposure to osteogenic factors in a particle-free environment. The inhibitory effects on differentiation are sustained even after particles are removed from culture.

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