PMMA Particles Can Activate Macrophages Independent Of Adherent Endotoxin
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Introduction: Aseptic loosening is the major cause of long-term failure of orthopaedic implants for joint replacement, resulting in approximately 40,000 revision surgeries annually in the United States. Wear particles released from the implant are phagocytosed by macrophages and stimulate the release of bone resorptive cytokines, leading to loss of implant fixation (1). The biological activity of debris particles may vary with their composition, size, morphology, concentration and surface adsorbed serum-derived proteins (2). Previous studies have focused on the role that adsorbed endotoxin, the lipopolysaccharide (LPS) from the cell wall of gram-negative bacteria, may play in the biological activity of wear particles. Some investigators have claimed that treatments which remove adsorbed endotoxin markedly decrease or completely reverse the biological activity of the particles (3,4); others have argued that the impact of these treatments is due to alterations in the particles themselves rather than to removal of endotoxin. Polymyxin B is an antibiotic used for treatment of gram-negative septic shock and works by binding and neutralizing LPS. The current study used polymyxin B treatment to bind and neutralize LPS as a means for determining the presence and significance of adherent endotoxin on the effects of wear debris particles.

Materials and Methods: Preparation of particles: PMMA particles were washed 5X with 70% ethanol and incubated overnight with shaking at 4°C. The particles were then washed 3X with DPBS and resuspended to a concentration of 16% v/v. The particles were free of endotoxin using a high-sensitivity Limulus amoebocyte lysate assay (BioWhittaker).

Polymyxin B preparation: Polymyxin B was prepared fresh before each use and added to cell cultures at a concentration of 10 μg/ml. This concentration has been shown to decrease LPS-induced inflammatory reactions without causing significant toxicity.

Raw264.7 macrophage cell culture experiments: The murine monocyte/macrophage cell line Raw 264.7 was cultured in DMEM containing 10% (v/v) FBS (5% CO2, 37°C). Cells were plated in 24-well tissue culture plates at 1 x 10^5 cells/well in 1 ml of media with serum and allowed to adhere for 24 hours. The media was then replaced with 1 ml of media containing one of four conditions: 1) media alone; 2) 10 μg/ml of polymyxin B; 3) 500 pg/ml of LPS (E. coli 055:B5, Sigma); 4) 500 μg/ml LPS and 10 μg/ml polymyxin B. TNF-α release was measured at 12 h after incubation using ELISA. Each group was plated in triplicate and the experiment was repeated two times. Data were analyzed by ANOVA.

Quantification of TNF-α release with wear debris particles: Macrophages were cultured as above. The media was then replaced with 1 ml of media containing one of three conditions: 1) media alone; 2) PMMA particles at a dose of 0.30% v/v; 3) 0.30% PMMA particles plus 10 μg/ml of polymyxin B. TNF-α levels were measured at 1, 4, and 12 hours post challenge.

Results: Macrophages incubated with polymyxin B for 12 h had no significant difference in TNF-α release compared to macrophages alone (Figure 1). The addition of LPS markedly increased TNF-α release. Polymyxin B markedly decreased the effects of LPS. LPS alone resulted in TNF-α levels above the upper range of the assay (2250 pg/ml). The decrease with polymyxin B to 1120 pg/ml therefore represents at least a 50% reduction in TNF-α release.

Macrophages incubated without particles or LPS had very low levels of TNF-α release. Macrophages incubated with PMMA particles alone had marked time-dependent increases in TNF-α release (Figure 2). The addition of polymyxin B to particles did not alter TNF-α release at 4 h and produced a non-significant 25% decrease at 12 h.

Discussion: This study using the Raw264.7 macrophage cell line demonstrated that the majority of the inflammatory response to PMMA particles is not simply attributable to endotoxin contamination but rather is due to the particles themselves. By neutralizing endotoxin, polymyxin B decreased LPS-induced TNF-α release in the absence of particles by at least 50%. In contrast, polymyxin B had no significant effect on particle-induced TNF-α release. The washing protocol for the PMMA particles used in this study appears to effectively remove any residual adsorbed endotoxin without altering the ability of the particles to activate macrophages. The exquisite sensitivity of macrophages to LPS (marked activation at LPS concentration of 500 pg/ml) is consistent with studies which suggest that LPS contamination may be responsible for macrophage activation in some particle experiments. However, by neutralizing LPS without needing harsh treatments to remove LPS, our results demonstrate that particles alone, without residual LPS, are sufficient to produce intense macrophage activation.