INTRODUCTION:

Joint implant wear particles are recognized to play a major role in initiating periprosthetic osteolysis leading to aseptic loosening and implant failure. It has been shown that particle composition, size, shape and concentration influence the inflammatory response to wear particles [1,2]. Metal-metal (MM) and ceramic-ceramic (CC) bearings are common alternatives to conventional metal-polyethylene bearings. While MM bearings produce relatively low wear, mainly in the form of 30-60 nm chromium oxide particles Effects of similar ly sized nanometer Cr2O3 and Al2O3 particles remain unknown. CC implants generate nanometer- to micrometer-size alumina particles [4], which have been shown to be more bioinert compared to conventional polyethylene particles [2]. The difference between the biological reactions to nanometer-size chromium oxide versus alumina particles remains to be investigated. Therefore, the purpose of this study was to analyze and compare the cytotoxic effects of similarly sized nanometer chromium oxide and alumina particles on macrophages in vitro.

METHODS:

J774 mouse macrophages (ATCC) were exposed to commercially available round 60 nm Cr2O3 particles (Sigma) or 50 nm Al2O3 particles (American Elements) in increasing concentrations (up to 3.5 million particles/macrophage). Particles were first sterilized for 1-3 hours in 70% ethanol, followed by 10 minutes in an ultrasonic bath to allow resuspension, and 3 washes in phosphate buffered saline solution. Half a million macrophages were used for each condition, resuspended in 1 ml of medium containing the particles, except for the negative controls (no particles). Incubations were conducted in tubes for 20-24 hours at 37°C, 5% CO2, and in a humidified environment, using a rotator for constant resuspension of the particles in the medium. Particle phagocytosis was observed using standard light microscopy and by analyzing changes in the forward scatter (cell size) and side scatter (index of cell granularity) using flow cytometry, as previously described [2]. Cytotoxicity was evaluated by measuring the remaining total cell number (both viable and dead) and cell mortality after macrophage incubation with the particles. The total cell number was measured by light microscopy using a hemacytometer. Cell mortality by apoptosis and necrosis was quantified by flow cytometry using an Annexin-V/propidium iodide (PI) assay (Trevigen) and by ELISA using a cell death kit (Roche) for differentiating late apoptotic from necrotic cell mortality, as well as TNF-α cytokine and MCP-1 chemokine release. Light microscopy results also showed that both Cr2O3 and Al2O3 particles were phagocytosed by the macrophages in a dose-dependent manner. Light microscopy results also showed that both Cr2O3 and Al2O3 particles caused a significant decrease in the total cell number with increasing particle concentration. Cell numbers decreased by up to about 70% and 25% compared to control with 3.5 million of Cr2O3 and Al2O3 particles/cell, respectively (p<0.02) (Figure 1). This suggests a higher cytotoxicity of the 60 nm Cr2O3 particles compared to the 50 nm Al2O3 particles in terms of absolute particle numbers.

DISCUSSION:

Cytotoxic effects of similarly sized nanometer Cr2O3 and Al2O3 particles on macrophages in vitro were analyzed and compared by measuring changes in the total cell number, increases and types of cell mortality, as well as TNF-α cytokine and MCP-1 chemokine release. J774 mouse macrophages were chosen because of their morphological and behavioral similarities with human macrophages at the implant site [5]. Results demonstrated that nanometer-size Cr2O3 and Al2O3 particles can have cytotoxic effects on macrophages, as shown by the decreasing numbers of total cells and a significant increase in cell mortality with increasing particle concentrations. Cells incubated with 60 nm Cr2O3 particles resulted in lower total cell numbers and higher levels of necrosis compared to cells incubated with similar numbers of 50 nm Al2O3 particles, suggesting higher relative cytotoxicity of the Cr2O3 particles. However, TNF-α levels remained low for both types of particles, demonstrating that overall, nanometer-size Cr2O3 and Al2O3 particles, two types of stable ceramics, induced a lower inflammatory response compared to previously reported levels with micrometer-size ceramic and polyethylene particles [2]. Effects on cell metabolism (e.g., inhibition of proliferation) induced by the different types of particles remain to be investigated.

REFERENCES:


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