Introduction:
Aseptic loosening following total joint arthroplasty results in part from chronic inflammatory reaction to particulate debris generated from implant biomaterials. The granulomatous tissue that forms in response to the wear particles contributes to localized osteolysis at the site of the implant. Numerous studies document the presence of abundant macrophages within the periprosthetic tissue and the proinflammatory mediators released by the cells are thought to accelerate bone loss. The macrophages are activated by various orthopaedic implant debris including polymethylmethacrylate (PMMA) particles. Particle activated macrophages exhibit NF-κB nuclear translocation that is accompanied by an increase in IL-6 and TNF-α production. The induction of transcription factor activation in response to particles also occurs in the macrophage response to bacterial lipopolysaccharide (LPS). The antiinflammatory cytokine, IL-10, suppresses NF-κB activation and IL-6 and TNF-α production by macrophages in response to LPS. However, tyrosine phosphorylation patterns between PMMA and LPS activated macrophages are different. The study addressed the hypothesis that human macrophage NF-κB nuclear translocation in response to PMMA will be suppressed by IL-10. This antiinflammatory cytokine, IL-10, suppresses NF-κB activation and thus IL-6 and TNF-α production.

Methods:

Macrophage Isolation: Buffly coats were obtained from healthy donors. The collected cells were diluted in Dulbecco’s phosphate buffered saline (DPBS) and a Ficoll-Paque (Pharmacia) fractionation was performed to isolate the mononuclear cells. Mononuclear cells were washed three times in DPBS and plated on 100 mm plates (Nunc) in RPMI 1640 (Gibco) supplemented with 5% human serum (Sigma) and 25 µg/mL gentamicin. After a sixteen hour adherence period the nonadherent cells were washed off and discarded. The adherent cells were harvested with trypsin, washed, and replated in RPMI 1640 supplemented with 5% human serum and 25 µg/mL gentamicin. Cells used for the cytokine assays were plated at a concentration of 5X10^5 in 24 well plates (Nunc). Cells used for the electrophoretic mobility shift assays (EMSA) were plated at a concentration of 6X10^6 on 100 mm plates (Nunc). IL-10 was added at stated concentrations 10 minutes prior to particle or LPS exposure. Particles: Commercially available PMMA particles (1-10 µm, mean size = 6 µm) were used for the particle challenged (PA) groups. The particles were sterilized in 70% ethanol, washed with DPBS, resuspended in serum free RPMI 1640, and added to the macrophages at a dose of 0.075% (v/v). Controls: Medium alone (CTL) was used as a negative control. Medium containing particles or LPS represented activating conditions.

Cytokine Assays: Medium was harvested 48 hours after particle addition. IL-6 and TNF-α concentrations were measured using commercially available ELISA kits (R&D Systems, Minneapolis, MN). Nuclear Extraction: After 48 min of exposure to PMMA or LPS with or without IL-10, nuclei were harvested as follows. After removal of the medium samples, each plate was washed with DPBS. Cold DPBS was added to each plate and the cells were scraped off and collected by centrifugation at 600 x g for 20 minutes in the cold (4 °C). The supernatant was discarded and the cells were resuspended in 1 mL of cold DPBS. The solution was transferred to a 1.5 mL Eppendorf tube and placed on ice. The rest of the procedure was performed in a cold room at 4 °C. The 1.5 mL tubes were spun at 14,000 rpm for 15 sec. The supernatant was removed and the pellet was resuspended in 400 µL of buffer A (10 mM HEPES pH 7.8, 10 mM KCl, 2 mM MgCl₂, 0.1 mM EDTA, 0.5 mM DTT, 0.5 mM PMSF). Cells were incubated on ice for 15 min. 25 µL of 10% Nonidet P-40 were added to each tube. The tubes were vortexed for 10 sec an then spun at 14,000 rpm for 30 sec. The supernatant was removed and the pellets were resuspended in 40 µL of buffer C (50 mM HEPES pH 7.8, 50 mM KCl, 300 mM NaCl, 0.1 mM EDTA, 0.5 mM DTT, 10% (v/v) glycerol, 0.5 mM PMSE). The nuclear proteins were then salt extracted on a rotator for 30 min.

Results:

Cytokine Data: IL-10 reduced the levels of IL-6 and TNF-α release by macrophages in response to PMMA particles in a dose dependent manner. At 48 hours, IL-6 release was inhibited by 60% and 90% at concentrations of 1.0 and 10.0 ng/mL of IL-10, respectively. At 48 hours, TNF-α release was inhibited by 58% and 96% at concentrations of 1.0 and 10.0 ng/mL of IL-10, respectively.

EMSA Data: IL-10 (10 ng/mL) does not suppress NF-κB activation in macrophages exposed to PMMA (Table I), and incompletely suppresses NF-κB activation in macrophages exposed to LPS. IL-10 (20 ng/mL) suppresses NF-κB activation in macrophages exposed to LPS, but not in those exposed to PMMA.

Discussion:

The data demonstrate that IL-10 inhibited the release of IL-6 and TNF-α in macrophages exposed to PMMA particles and that macrophage NF-κB activation in response to PMMA was less sensitive to IL-10 compared to a NF-κB activation in response to LPS. In the LPS model three transcription factors have been shown to be necessary for macrophage IL-6 and TNF-α synthesis: NF-κB, NF-IL-6, and AP-1. In the LPS model IL-10 suppresses NF-κB activation and thus IL-6 and TNF-α production. The data from this study indicate that IL-10 suppresses macrophage production of IL-6 and TNF-α in response to PMMA at 48 hours. However, IL-10 does not suppress NF-κB activation in response to PMMA within 90 minutes. The current data imply that during the first 48 hours in the PMMA model, IL-10 acts either by inhibition of transcription through alternative mechanisms or through post-transcriptional processes. These results are important to understanding the intracellular mechanisms by which particulate debris contributes to long-term, chronic periprosthetic inflammation.

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