Macrophage Cytokine Release in Response to Gelsolin in the Presence of Titanium Particles

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Introduction: Each year, the number of total joint arthroplasties performed to reduce joint pain from patients suffering from osteoarthritis continues to rise. However, the rate of revision surgery required to correct failed implants is rising at an even faster rate. A significant number of failed implants are a result of local tissue reaction to foreign implant debris that lead to osteolysis and aseptic loosening, but the mechanisms of these reactions are not fully understood. Gelsolin (GSN) is an abundant plasma protein that has been shown to regulate the assembly and disassembly of actin filaments of the cytoskeleton intracellularly, and is believed to serve a protective role in plasma by degrading cytoskeletal byproducts of cell death that may occlude the microcirculation or trigger an immune response. GSN has also been implicated as a biomarker for trauma, asthma, and rheumatoid arthritis patients. The effect of GSN in the local tissue reaction to implant wear particles has not yet been shown, but could play an integral part in understanding how GSN levels may be manipulated for therapeutic effect and used as a diagnostic predictor of implant success. This study used a human monocyte cell line to investigate how different doses of GSN with implant wear particles affect cytokine release. We hypothesize that GSN, in the presence of wear debris in vitro, will inhibit the release of pro-inflammatory cytokines and stimulate the release of anti-inflammatory cytokines.

Methods: A human monocyte cell line (THP-1, ATCC) was cultured in growth medium containing RPMI 1640 medium (Sigma) supplemented with 1% L-glutamine (Sigma), 0.13% gentamycin (Sigma), 0.05 mM beta-mercaptoethanol, and 10% fetal bovine serum (Sigma). Cells were cultured at 37°C with 5% CO2, and growth medium was replaced when cells were nearly confluent. Cells were differentiated into adherent macrophages using 100 nM phorbol myristate acetate (Sigma). 48 hours post-differentiation, cells were suspended in serum-free medium and transferred to a 24 well culture plate at 250,000 cells per well. Cells were exposed to different doses (0, 0.1 uM, and 0.2 uM) of GSN from two sources (Cytoskeleton; BioAegis Therapeutics) and either clean titanium alloy (Ti) particles (BioEngineering Solutions) or Ti particles coated with lipopolysaccharide (LPS, Invitrogen) at a ratio of 10 particles per cell. A second group of cells were exposed to GSN, clean Ti, as well as 5% human heat-inactivated serum (Sigma). After exposure, cells were incubated for 24 hours, after which the medium was removed and assayed for TNF-α, IL-6, IL-10, and IL-17 using ELISA (R&D Systems). Cells were then given fresh growth medium supplemented with MTS (CellTiter 96® AQueous Non-Radioactive Cell Proliferation Assay, Promega). After incubating for one hour, the absorbance was read and recorded to assess toxicity. Trials were performed in triplicate. ANOVA was performed for analysis of mean cytokine production with dosing trials of GSN and Student’s t-test was performed for comparison between positive and negative controls.

Results: Figure 1. TNF-α release (mean + SEM) from cells exposed to Ti particles and different doses of GSN from Cytoskeleton or BioAegis Therapeutics.
Figure 2. TNF-α release (mean + SEM) from cells exposed to different doses of GSN and the indicated conditions.
Figure 3. IL-6 release (mean + SEM) from cells exposed to different doses of GSN and the indicated conditions.

Consistent non-toxic cell viability was seen in all conditions (data not shown). The GSN from Cytoskeleton unexpectedly stimulated TNF-α secretion dose dependently (Figure 1), and was later confirmed to have endotoxin contamination by a Limulus Amoebocyte Lysate assay (data not shown). The endotoxin free GSN from BioAegis Therapeutics was used for subsequent experiments. LPS-coated Ti had an expected pro-inflammatory effect on cells, inducing TNF-α and IL-6 release. This effect was not mitigated by the addition of 0.1 or 0.2 uM GSN except for a slight decrease in IL-6 release observed in cells exposed to 0.2 uM compared to 0 uM GSN (Figure 2-3). GSN had no effect on background TNF-α or IL-6 secretion in cells exposed to clean Ti, and this behavior was not affected by the addition of human serum (Figure 2-3). There were only background levels of IL-10 and IL-17 release in all of the tested conditions, and these levels were also not affected by addition of GSN (data not shown).

**Discussion:** At the tested doses, GSN’s anti-inflammatory properties implicated by the literature were not observed in these experiments. GSN did not significantly decrease TNF-α or IL-6 secretion in a pro-inflammatory state stimulated by LPS except for a marginal decrease in IL-6 release when 0.2 uM GSN was administered. The tested doses are lower than typical serum levels (2 uM) for GSN, therefore a more pronounced effect at higher GSN doses seems promising. There was no observed effect on cytokine secretion when exposed to clean wear particles at the 10 particles per cell dose. GSN did not affect secretion of IL-17 or IL-10 in these experiments. Testing is ongoing to determine if anti-inflammatory effects can be observed at higher doses and using additional cytokines and wear particles (cobalt and polyethylene). Serum effects will also be assessed in future studies. GSN’s observed physiologic effects may not be limited to macrophage cytokine secretion, but may involve other cells. Future studies will also explore GSN’s effect on fibroblasts and neutrophils.

**Significance:** The effect of GSN in the local tissue reaction to implant wear particles has not yet been shown, but could play an integral part in understanding how GSN levels may be manipulated for therapeutic effect and used as a diagnostic predictor of implant success. This study used a human monocyte cell line to investigate how different doses of GSN with implant wear particles affect cytokine release.
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