Effects of Gelsolin on Macrophage Inflammatory Responses to Implant Wear Debris

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Introduction: Gelsolin is an abundant protein component of plasma whose function is not completely understood. Its role in actin cytoskeletal remodeling has been established and its intracellular production and gene are linked to extracellular GSN in the plasma. It has been suggested that GSN in the plasma serves to protect the micro-circulation from by-products of cytoskeletal elements released during the normal processes of cell death from pathological or traumatic causes. Gelsolin levels have been implicated as a prognostic value in trauma patients, as well as a substance that is present in asthmatic surfactant. In asthmatics it has been determined that IL-4 upregulates GSN which may enhance surfactant flow in airways of the lung in allergic asthma and aid in the clearance of cellular debris. An investigation has also determined that GSN in the plasma may aid in the inhibition of endotoxins in vivo and also has anti-inflammatory properties. In rheumatoid arthritis patients it has been shown that synovial fluid levels of GSN are significantly lower than in normal or osteoarthritic joint synovial fluid. To date no study has looked into the effects of GSN on the local response to wear particles from orthopaedic implants. If GSN is another marker of increased response to local implant wear particles and debris, then perhaps it can be utilized in targeting the inflammatory response locally to decrease the short and long term effects. This study utilized a human monocyte cell line to explore the effects GSN has on their response to different types of implant wear debris. The hypothesis was that in normal serum concentrations GSN would decrease the inflammatory response of monocytes while lower GSN concentrations would allow a larger inflammatory cytokine release from the monocytes.

Methods: A human monocyte cell line (THP-1) from ATCC was cultured in growth medium containing RPMI 1640 medium (Sigma) supplemented with 1% L-glutamine (Sigma), 0.13% gentamicin (Sigma), 0.05 mM mercaptoethanol and 10% fetal calf serum (FCS) (Gibco). These cells are phagocytic for both latex beads and sensitized erythrocytes. Cells were initially grown in 25 cm2 flasks (Costar) and cultured at 37°C, with 5% CO2. Growth medium was changed every 2-3 days, and when nearly confluent (usually 10 days after the last passage) cells were subcultured. They were differentiated by PMA (phorbol myristate acetate) 48 hours prior to the experiment. The cells were exposed to either UHMWPE, Ti and Co particles (BioEngineering Solutions Inc.) for 24 hours in 24 well culture plates at a density of 200,000 cells per well in triplicate. A fraction of the particles were incubated in 0.78 µg/ml endotoxin (invitrogen) at room temperature for 24 hours, and then washed with endotoxin-free water 5 times to remove the unbound endotoxin and used as positive controls. Gelsolin was added to appropriate wells at 0.2 µM and 2.0 µM. Gelsolin as well as all particle dilutions were done in serum-free medium except for PE particles which required serum to go into solution. Particle to cell ratios of 100:1 and 500:1 were used in the experiment. Positive control particles (LPS-exposed) were used at 100 particles/cell. After 24 hours of incubation at 37°C with 5% CO2, the medium was removed and assayed for TNFa, IL-1β, IL-6 (R&D Systems) and PGE2 (Assay Designs Inc.). A cell viability test was done using “Cell Titer 96® AQueous One Solution Cell Proliferation Assay” (Promega).

Results: Good cell viability was seen in all cases except for 500:1 titanium particle group. Gelsolin did improve cell viability with both groups of 100:1 and 500:1 particles of Cobalt and with 500:1 particles of titanium. Gelsolin had a pro-inflammatory effect in all cases as seen with TNF-α, IL-6, and IL-1β. TNFalpha levels with LPS and GSN showed that lower levels of GSN decreased TNFalpha levels without the addition of implant particles. This was seen with all levels of particle concentrations except in the case of polyethylene particles. For IL-6 the same trends as described for TNFalpha resulted with lower IL-6 levels at lower GSN concentrations except in the case of polyethylene particles. Similar results for PGE2 were also seen. However, macrophages exposed to cobalt particles secreted PGE2 with or without the presence of either low or normal Gelsolin concentrations.

Discussion: Our results show that in serum free culture, Gelsolin appears to induce secretion of pro-inflammatory cytokines. Although our hypotheses were not validated in this study, the question remains as to whether there is a serum binding effect of GSN that would explain these results. Since the lower concentration of GSN had a lower response of IL-1β, IL-6, TN alpha and PGE2, it suggests that without the presence of serum to bind the GSN (as happens in vivo), the actual effective amount of GSN exposure in this experiment may be significantly higher and thus eliciting a different response. We do plan on repeating our results with human serum to determine if this is the case as well as to test a much lower level (0.05 µM) of GSN. Although this is a preliminary study, the results do add to the knowledge of the effects GSN has in the presence of implant wear debris. The dose effect curve needs to be mapped out for lower concentrations as well as the serum binding effects of GSN in the in vitro setting.

Significance: If gelsolin effects the response of macrophages to implant wear debris in vitro then it may be able to be utilized as
a marker for osteolysis or as target to prevent osteolysis in vivo.

Acknowledgments: NA

References:

![Cell Viability](image)

**Figure 1:** Cell viability as measured for each wear particle tested.
Figure 2: TNF alpha levels for each wear particle and condition tested.
Figure 3: IL-6 level measured for each wear particle and condition tested.
Figure 4: PGE2 levels for each wear particle and condition tested.