ADSORBED ENDOTOXIN MEDIATES DIFFERENTIAL EFFECTS ON PARTICLE-INDUCED STIMULATION OF CYTOKINE AND CHEMOKINE RELEASE

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Background: Studies have demonstrated the capacity of metal wear particles from orthopaedic implants to stimulate the release of a variety of pro-inflammatory products. Little is known, however, concerning the mechanisms by which particle attachment and internalization lead to cell activation. Among the factors that influence reactivity are the intrinsic physical chemical properties of the particulate species but also the capacity of the particles to bind serum factors and to present them to cells. The cell responses are then transduced via interactions between these factors (opsinins) and specific cell surface receptors. Ragab et al (ORS; 355,1998) have recently reported that adsorbed bacterial endotoxin is the major factor responsible for actviation of cells by titanium metal particles. In the present studies we confirm these observations and further investigate the differential capacity of particles to bind endotoxin and induce distinct patterns of cytokine and chemokine responses.

Materials and Methods:

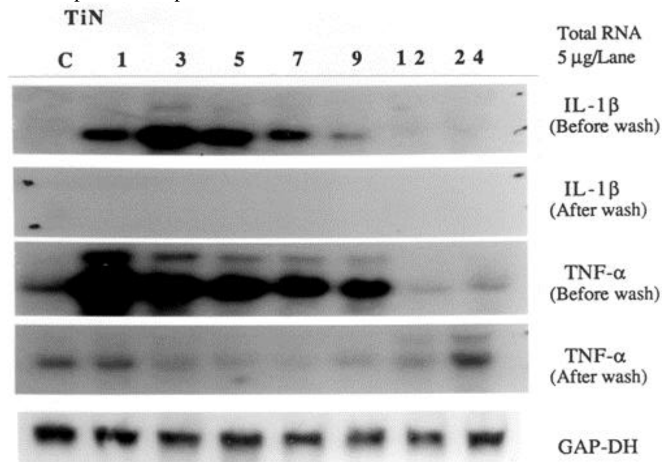
Particles: Silica (SiO_2) (avg. diam.=1.40 μm) (Corning) or titanium nitride (TiN) (avg. diam.=1.25 μm) (Alpha Chemicals) were prepared for culture by gas sterilization (H_2O_2). To inactivate particle-associated LPS, particles were boiled in 1% HAC for 90 min or heated in an autoclave for 1h at 260^0C.

Cell culture: RAW 264.7 and J774A.1 cells (murine macrophage cell lines; ATCC, Rockville, MD) were plated at $2x10^6$ in six-well tissue-culture trays (Costar) and cocultured with particles based on particle number distribution assuming a perfectly spherical particle. Human peripheral blood monocyte/macrophages (M/M) were isolated by Ficoll-diatriazoate gradient separation and adherence depletion. LPS (*E. coli* serotype 055:B5; Sigma) was diluted to appropriate concentrations prior to addition to cell cultures. Concentrations were confirmed by limulus assay (Biowhitaker; Walkerville, MD).

Assays: Cell proliferation and viabilty were assayed by MTT (Sigma). Detection of mRNA species were assayed by Riboquant Multi-Probe Rnase Protection Assay (RPA) (PharMingen) after Trizol extraction or by Northern blot analysis after lithium chloride extraction. TNFα, IL-1α, and IL-1β were assayed by ELISA (Genzyme).

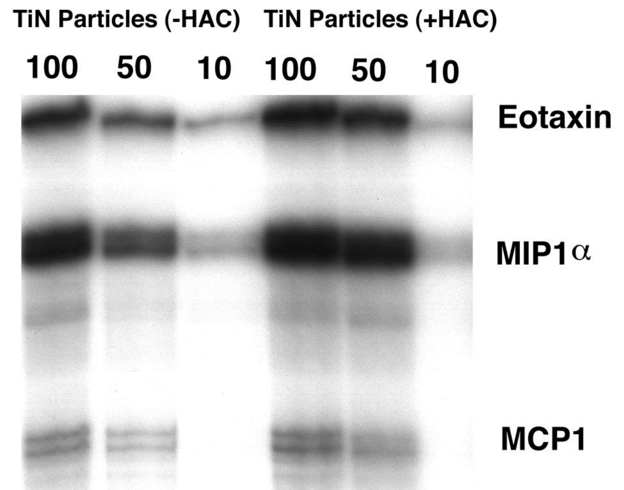
Results:

Addition of TiN or Si particles produced an increase in steady state IL-1 and TNF mRNA levels as assessed by Northern analysis. HAC treatment completely abolished TiN-induced increases in IL-1 mRNA levels and markedly diminished the TNF responses. These findings suggest that the major component of TiN particle-induced increases in cytokine mRNA levels were dependent on particle associated endotoxin.



Similar effects of HAC treatment on particle-mediated stimulation of cytokine release were observed with human M/M. Treatment of TiN abolished the IL-1 response. In contrast, HAC treatment of Si particles only partially abbrogated the cytokine response.

To further establish that the cytokine responses were dependent on particle-associated endotoxin, particles were preincubated with LPS (1000ng/ml) for 48h. After extensive washing, particles were treated with HAC or autoclaved to inactivate adsorbed LPS. LPS-treated particles produced >7 fold increase in TNF release from J774 cells. HAC treatment resulted in ~30% decrease in cytokine response and autoclaving almost completely abolished the particle-induced increase in TNF levels. In contrast to the effects LPS inactivation on IL-1 and TNF release, HAC treatment did not downregulate the effects of Si or TiN particles on chemokine (eotaxin, MIP1a, and MCP 1) steady mRNA levels as assessed by RPA.



Discussion: These studies provide further evidence that metal (and silica) particles have the capacity to induce cytokine and chemokine release from macrophage-like cells. These products provide a likely mechanism for the development of peri-implant granulomatous inflammation and accompanying osteolysis. Our findings confirm the previous observations made by Ragab et al and indicate that a significant component of the capacity of particles to induce macrophage activation is dependent on particle-associated endotoxin. Bacterial endotoxin has been shown to have a high affinity for metal implant materials, especially titanium and titanium alloy, and contamination of metal implant surfaces with LPS *in vivo* could result in activation of inflammatory cells at implant sites and contribute to local inflammatory reactions. The transformation of bulk implant materials into particulate species by wear mechanisms could provide a drastically increased surface area for LPS presentation to resident cell populations and markedly enhance the capacity of wear particles to induce proinflammatory cytokine release. In contrast to the effects of LPS-treated particles on IL-1 and TNF responses, the stimulatory effects of the particles on chemokine release did not appear to be LPS-dependent, indicating the presence of differential mechanisms for particle-mediated effects on cells responses.

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