Estimation of Biological Potential and Binding Affinity of Orthopaedic Wear Debris Bound to Different Endotoxins for Aseptic Loosening Pathogenesis Study

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Introduction:

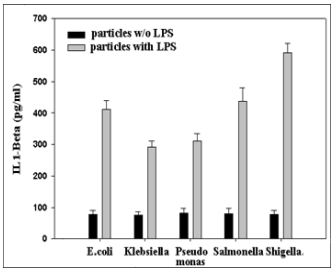
More than 300,000 total joint arthroplasties are performed in United States each year. However, 30% to 35% of implant recipients eventually fail due to chronic regional inflammation. It is likely the orthopaedic wear debris particles are able to provoke regional inflammatory response despite the development of improved orthopaedic procedures and materials. Indeed, many recent observations suggest that binding of bacterial endotoxins to particulate particles significantly enhances cellular reponses and products of inflammatory mediators. Although both sterilized surgical procedure and implant material are reliable, the remaining question is if the bacterial endotoxin such as lipopolysacchrides (LPS) binds to long-time existed wear debris particles. Most experimental studies documented that LPS is not washable with physiological normal saline from the LPS mixed particles. In order to further understand the mechanism of LPS binding to the particles, we investigated that the hypotheses (1) the differences of binding affinity of LPS to particulate ultra high weight polyethylene (UHMWPE) depend upon the biological characters of different derived from deferent bacterial strains; (2) UHMWPE particulate is a capable "core" that accumulates LPS in vivo after multiple endotoxin inoculations. The designed experiments mimic wear debris responses to LPS exposure in the body which may be seeded by one of the gram negative bacteria such as *E.coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Salmonella enterica* and *Shigella*. The experiments also reflect the biological binding potential of particles to endotoxins that may be a fundamental prerequisite to understand the pathogenesis of the wear-debris induced periprosthetic osteolysis. The outcomes of this study provide evidence that suggest both particles and endotoxin should be considered in the pathogenesis of particle-induced aseptic loosening.

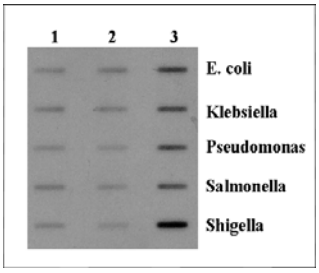
Materials and Methods

Materials. LPS from different bacteria strain (*E.coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Salmonella enterica*) was purchased from Sigma-Aldrich Co. Cytokine ELISA tection kits were purchased from R & D Systems. particles ultra high weight polyethylene (UHMWPE) a generous gift of Dr. John Cucklar from University Alabama, Birmingham AL. *Cell cultures.* For human monocytic cell cultures, peripheral blood from healthy human donors was used. The described techniques [(1)] were used to prepare monocytic cultures. Mouse macrophage cell line (RAW 264.7 purchased from ATCC) was also used for this study. *Analysis of particle bioactivity before and after mixed with endotoxin.* Particles were washed 3 times with ethanol and tested for LPS using a commercial detection kit (Limulus Amebocyte Lysate assay kit, Endosafe SC). Sterilized particles were split into two identical groups. One was suspended (30ug/ml) in the culture medium (MEM) which is used to culture human peripheral blood monocytic cells. Another was mixed with LPS (1:0.1 w/w) in normal saline for 90 min at room temperature. After, particles were washed 3 times (1:20 v/v) with phosphate buffer saline (PBS), particles were suspended (30ug/ml) in MEM prior use for experiment. After 24 hours 37^0C incubation, the particle contained solutions were added to monocytic cultures. After 48-hours incubation, total cytokine (extra and intra cellular) concentrations were estimated by ELISA assays. *Estimation of binding affinity of particle to LPS in the animal air pouch model.* BALB/c mice were purchased from The Jackson Laboratory, and housed for 7 days prior to use for experimental studies. The techniques used to induce air pouches were previously described [(2)]. Briefly, to establish an air pouch, 10- to 12-week-old mice were injected subcutaneously on the back with 3 ml of air. Every other day, the pouches were reinflated with 1 ml of air for 6 days. Sterilized particles were induced by injection of 1 ml of a suspension of 5% (wt/vol) UHMWPE in PBS into air pouch, as we have previously described [(2)]. One day after, mice were divided into two groups and treated for 3 consecutive days with either 0.2 ml of saline (control), or 0.2 ml of saline containing LPS (2ng/ml) by tail vein injection. 24 hours after last injection, all mice were sacrificed; the pouches were dissected under dissection microscope. Particles were collected by passing the pouch tissue samples through stainless steel grids in PBS. Tissues and particles were separated by gravity centrifugation. *Analysis of particle/LPS aggregates from air pouch tissue was done using immunoprecipitation.* House reddish peroxidase linked anti-LPS monoclonal antibody (IgG2a) was used to detect the LPS from particles. The described immunoprecipitation procedures were routinely used method in our laboratory [(1)]. The protein G beads precipitated antibody-LPS complexes were visualized using either diaminobenzidine development or ELC detection after electrophoresis.

Results

Comparing the bioactivities of particles with particle/LPS aggregates, the LPS contaminated particles significantly promote the production of IL-1β by human monocytic cells.(Left panel). The ELISA results also indicate that the LPS extracted from *Shigella* is a more powerful stimulator of IL-1β production from cultured cultured cells than LPS derived from the other listed bacterial pathogens. Because of deep





rinsing, it is reasonable to believe LPS binding to the particles dependent on its chemical interaction rather than electronic polar physical connection. The results from a further *in vivo* experiment showed particles are able to cumulate LPS. Results from the modified dot blot study (right panel: lines 1 to 3 represent animals that received no-LPS, one-time LPS and 3 times LPS tail vein injections respectively) indicate the amount of LPS were cumulated on the surface of particles.

Discussion

The present study demonstrates that orthopaedic wear debris UHMWPE or PMMA particles have tremendous potential to bind to the LPS. Reported results have shown that the LPS contaminated particles enhance monocytic inflammatory cytokine release. LPS-induced inflammatory cytokine expression depends on the monocytic membrane CD14 and TLR4 receptors. Both CD14 and TLR4 expressions are significant in phagocytic cells including monocytes, macrophages as well as multinuclear Langerhans' cells. Actually, these cells are responsible for the particle-induced regional inflammations. Because of the lack of CD14 internal domain, TLR4 signal pathway is predominant for the LPS-triggered cellular immunoresponses. In this study, by using LPS derived from various bacterial strains mixed with particles, we found that the bioactivities of the LPS on stimulation to monocytes are different between the gram negative bacterial strains. LPS is not washable with normal saline while the LPS/particle aggregation forms. The stimulation strength of LPS/particle aggregates on monocytes may rely on each LPS binding affinity to the particles and biological characters. For instance, the cell stimulation of LPS from *Shigella* is stronger than *Salmonella* and *E.coli* on monocytes. The most interesting result from the in vivo studies is that inoculated particles have the capacity of accumulating aggregates of LPS. The data indicates the extra cellular particles in the body are able to aggregate and accumulate the LPS. Taken all results together, LPS may be an unignored pathogen and in the presence of particles can cause aseptic loosening.

References

[(1)] Hao HN et al (2001) J Neurochem. 77(4):1027-35.
[(2)] Ren WP, Hao HN et al (2006) J Orthop Res. 24(8):1575-86.