THE ROLES OF HEAT SHOCK PROTEIN 60 AND TOLL-LIKE RECEPTORS IN THE RESPONSE TO WEAR DEBRIS PARTICLES IN ASEPTIC LOOSING

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INTRODUCTION

Aseptic loosening is characterized by chronic inflammation in peri-prosthetic tissue that often leads to implant failure, and is frequently provoked by the presence of wear debris. Wear debris retrieved from peri-prosthetic tissue is a heterogeneous mixture of materials with various sizes and shapes. The local interaction of cellular components with wear debris particles is a critical feature in the pathogenesis of aseptic loosening. One accepted hypothesis is that osteolysis occurs due to chronic expression of inflammatory cytokines such as TNF and IL-18 in inflammatory cells stimulated by particle contact. Although many studies suggest that the severity of osteolysis is related to the amount, size and shape of particles, precisely how the cells interact with wear debris and produce inflammatory mediator remains largely unknown. We hypothesize that wear debris particles may activate the monocyte Toll-like receptor (TLR) 4 signal pathway to produce inflammatory cytokines and heat-shock protein 60 (Hsp60). Cells produce Hsp60 not only to maintain their biological function, but also to protect themselves from infection and non-specific physiological stress, although Hsp regulation of cellular responses remains controversial. However, TLR4 has been found to be an additional receptor for Hsp by many recent studies. In this study, we have demonstrated both intracellular and extracellular forms of Hsp60 produced by monocytes stimulated in vitro with UHMWPE debris particles. Using commercial Hsp60 and anti-TLR4 mRNA antisense, we further demonstrated that monocyte production of IL-18 and TNF-a depended on TLR4 expression. These results suggest the new discovered particle-Hsp60-TLR4 signal pathway may be a additional mechanism to explain how the particle induces aseptic loosening pathological process. This finding may provide new therapeutic strategies to prevent and treat particle related osteolysis.

MATERIALS AND METHODS

Monocytic cell isolation and cell culture. The present study is part of an ongoing research protocol that has been approved by the Wayne State University School of Medicine Human Investigations Committee. Informed consent was obtained from all participants. Macrophage/monocytic cultures were prepared from peripheral blood monocytes previously described (Zhao et al. 2002). Briefly, mononuclear cells were harvested after Ficoll-paque gradient centrifugation. Further purification was performed using anti-CD68 antibody and MACS magnetic cell purification. Cells were seeded at 5 x 10^5 cell in T-25 tissue culture flasks and incubated for 10 days. Monocytes were harvested and reseded in 35 mm culture dishes in DMEM culture for 20hr and then washed two times with phosphate buffered saline (PBS) to remove non-adhesive cells. Three days after reseeding the phenotype of cultured cells was determined by immunocytological staining.

Detection of CD68, CD14 and TLRs expression. RT-PCR was used to detect gene activation of CD68, CD14 and TLR. Total cellular RNA was prepared from monocytic cultures stimulated with or without particles for various times. Primers were used as follows: TLR-2, forward primer 5'-GGCAGCAGAATACTACTTTGTG-3' and reverse primer 5'-CCAGTTAGTTCCGGTGTTCA-3'. For TLR-4 and TLR-6, forward primers 5'-GTCGTTCAGTGTCAGCTAGATAGG-3' and reverse primers 5'-GGTTTCCCATCGAGGAGGAG-3' were used to determine the TLR expression. Anti-human TLR2, 4, 6 and Hsp60 polyclonal antibodies (Santa Cruz Biotechnology, Inc. Santa Cruz, CA) were used to localize the positive cells and detect protein.

Antisense & I'm treatment. TLR4 antisense sequences were designed based on the information from the GeneBank database. The oligonucleotides were synthesized by Biognostik. Cell cultures were treated with chimeric or scrambled control oligonucleotides (0-10 μg/ml) for 18 hours prior HSP60 or particle added.

RESULTS

Stimulation with UHMWPE particles induced monocyte cultures to produce Hsp60 which was subsequently released into the culture medium (Figure 1). Using immunostaining and confocal microscopy, studies revealed that mononuclear cells strongly expressed Hsp60 after particle exposure (Figure 2). This suggests that Hsp60 not only regulates cell function through intracellular signals, but also raises the possibility of a direct contribution to cellular responses via a secretory form of Hsp.

DISCUSSION

These results suggest that particles can stimulate mononuclear cells by direct physical contact and induce a stress-related response. The stress activated cells produce Hsp60 both within the cytoplasm and in a secretory form. Secreted Hsp60 may subsequently activate cells by either autocrine or paracrine mechanisms. Moreover, UHMWPE particles can induce an elevated expression of TLR4, which supports the concept that this highly conserved pattern receptor may recognize synthetic polymers, in addition to naturally occurring bacterial polymers such as LPS. TLR4 positive cells produce high levels of cytokines that are related to osteolysis and aseptic loosening. This study describes for first time how the UHMWPE interacts with target cell by a particle-Hsp-TLR dependent pathway, and may provide novel insights into the relationship between biomaterials and cellular activation.