Damage-associated Molecular Patterns (damps) Of Toll-like Receptors In Aseptic Loosened Total Hip Arthroplasty

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Introduction: Total hip arthroplasty (THA) is a procedure that reliably provides pain relief and improves activities of daily living for patients suffering from destructive end stage hip joint disease. One important long-term complication of THA is aseptic loosening often combined with osteolysis. This is caused by foreign body inflammation against adhesive and abrasive wear particles and by adverse reactions against metal ions formed as a result of corrosion. The pathogenesis of aseptic peri-prosthetic loosening/osteolysis represents host reaction to the implant-derived debris. We focus on the innate immune responses in host. Toll-like receptors (TLR) form a family of innate immune receptors. TLRs are trans-membrane proteins of inflammatory/resident cells, which recognize infectious threat signals, so called danger signals. Differentiation of self from non-self (pathogen-associated molecular patterns) was earlier regarded as a unique property of the immune system. However, TLRs recognize also self-components (damage-associated molecular patterns: DAMPs) [1, 2], which may induce production of osteolytic inflammatory cytokines found in aseptic loose interfacial membrane. As inflammatory danger signals play important roles in innate immune responses and foreign body reactions, the present work was performed to assess the eventual presence and response of some key TLRs in the so called aseptic loosening. The presence of TLRs would indicate enhanced responsiveness of peri-prosthetic interface membrane to whatever TLR ligands might be implanted together with the sterile joint prosthesis in the primary operation or whatever ligands of non-self and/or self might later be gain access to peri-implant tissue.

It was hypothesized that necrotic and/or activated macrophages and other cell types release DAMP-like TLR ligands into extracellular matrix, where they might play potent roles as cytokines and stimulate aseptic peri-prosthetic loosening/osteolysis of THAs. The aim of this study was to analyze expression of DAMP-ligand for TLRs expressed around aseptically loosened THA implants.

Methods: Synovial-like membranes and regenerated capsular tissues were obtained from aseptically loosened THAs (n=15). Osteoarthritic synovial tissues samples (OA) without any marked inflammatory reaction were obtained at primary THA and used as control tissues (n=7).

Immunohistochemical analysis

Conventional immunochemical study was performed for TLR2, TLR4 (Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA), high mobility group box-1 (HMGB-1, Epitomics, Inc., Burlingame, CA, USA), heat shock protein (HSP) 27, TNF-α, IL-1β (Santa Cruz Biotechnology). Co-localization of HMGB-1, HSP27, and CD68 (Dako Cytomation, Glostrup, Denmark) and/or 5B5 (Santa Cruz Biotechnology, Inc.), were
examined by immunofluorescence staining method using Alexa fluorescent system (Molecular Probes Inc., Eugene, OR, USA).

**Results:** Immunohistochemical analysis

In aseptic synovial-like membrane, increased immunoreactivities to TLR2, TLR4, HMGB-1, HSP27, TNF-α, and IL-1β were mainly observed in CD68 positive macrophages. Localization of HMGB-1 was usually seen in the cytoplasm and occasionally pericellular matrix in periprosthetic tissue close to osteolytic lesions. Immunoreactivity to HMGB-1 was sometimes observed in macrophages. Also, immunoreactivity to HSP27 was sometimes observed in macrophages (Figure 1). On the other hand, in osteoarthritic synovium, only weakly TLR2, TLR4, HMGB-1, HSP27, TNF-α, and IL-1β labelled cells were observed in the lining, sublining stroma and perivascular infiltrates. In osteoarthritic synovium, nuclei were usually HMGB-1 negative, but occasionally HMGB1 was seen in the cytoplasm.

**Discussion:** This study is first report of endogenous ligands of TLR2 and 4, relating with periprosthetic aseptic and osteolytic loosening.

HMGB-1 was first described as a member of the non-histone DNA-binding proteins [3]. It has been recently discovered that HMGB-1 is passively released by necrotic cells and actively secreted by a variety of cells in response to inflammatory stimuli [4, 5]. Reduced HMGB1 combines with CXCL12 and acts as a chemoattractant to cells such as endothelial cells, macrophages, neutrophils and osteoblasts [6, 7]. Disulfide-bonded HMGB-1 is a pro-inflammatory cytokine [8]. Cytoplasmic and/or extracellular HMGB-1 has been described in experimental arthritis and synovial fluid and tissues in rheumatoid arthritis [6, 9].

HSPs are well-preserved and present in all organisms and in all cells of all organisms and accumulate in cells exposed to heat and a variety of other stressful stimuli. Recently, HSPs have been implicated in antigen presentation. In addition, extracellular HSPs can stimulate professional antigen-presenting cells of the immune system, such as macrophages and dendritic cells. The events of cell stress and cell death are linked and HSPs induced in response to stress appear to function at key regulatory points in the control of apoptosis. HSPs include anti-apoptotic and pro-apoptotic proteins that interact with a variety of cellular proteins [10, 11].

Expression of HMGB-1 and HSP27 was confirmed in aseptic peri-prosthetic loosening/osteolysis. HMGB-1 and HSP27 are known TLR ligands. HSP27 are important in cell death and activation of signal pathways. Once these DAMPs are produced/secreted into extracellular matrix, they stimulate cell differentiation in an autocrine, paracrine or endocrine manner. Aseptic tissues around THAs are well equipped with TLR2 and TLR4 in numerous macrophages, which finding suggests enhanced responsiveness to TLR ligands even in the process of aseptic foreign body type reaction. The bone-implant interface is continuously exposed to byproducts of wear debris, pressure changes, and microbial products. The biological host reactions to such stimulation constitute foreign body granuloma (particle disease), pseudotumors (ion disease) and chronic inflammatory response. In this process, resident cell surrounding with implants result in necrosis and inflammatory reaction. Not only HMGB-1 but also HSP27 is recognized by TLRs. Both are produced by macrophages and fibroblasts and play potent roles of peri-prosthetic aseptic loosening/osteolysis and activate the inflammatory cascade via TLRs. Such events induce not only production/secretion of inflammatory cytokines, but also activation of matrix metalloproteinases. These functional proteins are important mediators for the pathogenesis of aseptic peri-prosthetic loosening/osteolysis.
Once DAMPs are released in host, they may directly induce prolonged cell reactions, chronic inflammation, and indirectly promote degradation of extracellular matrix proteins and promote cell invasion. Host-derived DAMPs are likely to contribute to the pathogenesis of aseptic peri-prosthetic loosening/osteolysis as one of the two major categories of danger signals.

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Significance: Aseptic loosening and peri-prosthetic osteolysis are critical long-term complications of THAs. Understanding and modulation of the innate immune system response to biomaterial byproducts from THAs may potentially mitigate the adverse reaction by the bone and surrounding tissues to the byproducts of implant wear.