**Different Profiles of Toll-like Receptors (TLRs) Expressions in Aseptic Loose Periprosthetic Tissues and Septic Synovial Membranes around Total Hip Implants.**

**INTRODUCTION:**
Total hip replacement (THR) is a procedure that reliably provides pain relief and improves daily activity for patients suffering from destructive end stage hip joint disease. The main long-term complication of THR surgery is prosthesis loosening often combined with osteolysis. The causes of loosening are mainly divided into two major categories. One is the septic loosening caused by implant infection and the other one is the so called aseptic loosening caused by foreign body inflammation against adhesive and abrasive wear debris and by delayed-type hypersensitivity reaction against self modified by metal ions formed as a result of corrosion.

Toll-like receptors (TLRs) are mammalian homologue of Toll receptor, which was originally identified in Drosophila [1]. TLRs are transmembrane proteins of inflammatory cells and essential players in the recognition of microbial (pathogen-associated molecular patterns; PAMPs, dead or alive microorganisms) and endogenous (alarmins) signals [2], which together form the so called “danger signals”. Endogenous alarmins and exogenous PAMPs can be considered subgroups of a larger set, the damage associated molecular patterns (DAMPs) [3]. They stimulate inflammatory responses via TLRs, but provide also the danger signal to adaptive immune responses, which may induce production of osteolytic inflammatory cytokines found in aseptic loose interfacial membrane. As inflammatory molecules play important roles in not only innate immune responses, but also 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 and septic artificial hip joints.

**Materials and Methods:**
Synovial-like membranes and regenerated capsular tissues were obtained from aseptic (n=15) and septic (n=5) loose total hip joints. Osteoarthritic synovium without marked inflammatory reaction was obtained at primary THR and was used as control tissues (n=5).

1) **Immunohistochemical analysis:**
Conventional immunohistochemical study was performed for TLR1, 2, 3, 4, 5, 7, and 9. Co-localization of TLRs, CD68 (a specific marker of monocytes/macrophages) and/or CD15 (a specific marker of neutrophils/monocytes), were examined by immunofluorescence staining method using Alexa fluorescent system (Molecular Probes Inc., OR, USA).

2) **Reverse transcriptase polymerase chain reaction (RT-PCR) and quantitative real-time PCR:**
Total RNA was isolated from each frozen tissues, following to convert into cDNA and enzymatic amplification of specific cDNA sequences was performed on Light Cycler system (Roch. Germany). TLR1, 2, 3, 4, 5, 7, and 9 mRNA were amplified. Quantitative analysis of the mRNAs was performed with the use of Light Cycler Software at each sample. β-actin, house keeping gene, was also amplified as internal control.

**RESULTS:**
1) **Immunohistochemical analysis:**
CD68 positive cells were observed in focal stromal monocyte/macrophage infiltrates and synovial lining cells both in the interface and regenerative capsular tissues from aseptically loosened hip joints. TLRs positive cells were also seen in the monocyte/macrophage infiltrates and in the synovial lining cells both in the interface and regenerative capsular tissues retrieved from aseptically loosened hip joints. Co-localization of TLR1, 2, 3, 4, 5, 7 and 9 with CD68 was confirmed by immunofluorescence staining (Fig 1). In septic loosened artificial hip joints, CD15 positive cells were observed in focal neutrophil/monocyte infiltrates, which were co-localized with each TLR. In osteoarthritic synovial membrane, a few scattered CD68 positive cells were observed in the synovial lining and sub-lining layers and perivascularly. Expression of TLRs in the osteoarthritic synovium was only found in vascular cells and the reactivity was weak.

2) **Quantitative real-time PCR:**
Expression of TLR1, 2, 3, 4, 5, 7, and 9 was detectable in all the samples from three different status of pathology. Increased expression of TLR2, 4, 5, and 9 mRNA level in aseptic and septic tissues around total hip joints was evident when compared with that of osteoarthritic synovium without marked inflammatory reaction (Fig 2). mRNA levels of TLR1, 3, and 7 in aseptic tissues and osteoarthritic synovial samples was lower when compared with that of septic tissues.

**Discussion and conclusion:**
Immunoreactivity of each TLR was mainly CD68 positive monocyte/macrophages and synovial-like membrane in aseptic tissues, and CD15 positive neutrophils/monocytes in septic tissues, respectively, around THR joints. Both tissues equipped TLRs, but the degree of expression was different. mRNA analyses suggested hyper-responsiveness to exogenous PAMPs of microbial origin and/or endogenous alarmins stimuli via TLR1, 2, 3, 4, 5, 7 and 9 in septic tissues, whereas, aseptic tissues were characterized by hyper-responsiveness via TLR2, 4, 5, and 9, but modest one via TLR1, 3 and 7. It was probably dependent on the type of infiltrating cells, which would play an important role in chronic reaction of foreign body type granuloma in aseptic tissues, and in acute/subacute reaction to microbial enemies in septic tissues. It was peculiar finding that increased expression of TLR2, 4, 5 and 9 was found not only in septic but also in aseptic tissues, but its biological interpretation was unclear. Responsiveness to endogenous alarmins signals of monocyte/macrophages in asptic foreign body type granuloma or circulatory latent microbial components may relate to the increased expressions in-situ, thus contribute to enhanced production of osteolytic inflammatory cytokines and to implant loosening. The further analysis would be required to address on the issue.

**REFERENCES:**

**Figure 1.** Co-localization of TLR5 and CD68 in synovial-like membrane around aseptic loosening/osteolysis. A: TLR5 was red, B: CD68 was green, C: merged photo is yellow.

**Figure 2.** Quantitative analysis, mRNA expression levels of TLR5 was shown. Graphs showed ratio to aseptic and septic tissues around artificial hip joints versus osteoarthritic tissues. *: p < 0.05.