TISSUE LOCALIZATION AND POSSIBLE FUNCTIONAL ROLE OF TOLL-LIKE RECEPTORS (TLRs) IN LOOSE TOTAL HIP JOINTS

INTRODUCTION:
The causes of loosening are mainly divided into two major categories. One is the septic loosening caused by implant infection and the other is the so-called aseptic loosening caused by foreign body inflammation against adhesive and abrasive wear debris. It has lately become apparent that even dead microbes, not able to divide, can provoke inflammation as ligands for toll-like receptors (TLRs). TLRs are transmembrane proteins of inflammatory cells, which recognize infectious threats, so called danger signals. Differentiation of self from non-self was earlier regarded as a unique property of the immune system. Recently, it became evident that TLRs can also recognize the molecule of self-components, 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. The presence of TLRs would indicate enhanced responsiveness of periprosthetic 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 components might later be absorbed from the circulation to the surface of the implant or implant-derived wear debris.

MATERIALS and METHODS:
1) Immunohistochemical analysis; Synovial-like interfacial membrane between bone and implants and the regenerated capsular tissues of loose hip prosthesis were obtained at revision total hip arthroplasty (n=10). Co-localization of TLRs and CD68, a specific marker of monocytes/macrophages, was examined using Alexa fluorescent system (Molecular Probes Inc., OR, USA). Osteoarthritic synovial tissues were used as controls (n=5).

2) Culture of monocytes/macrophages with particle stimulation;
Bone marrow cell suspension of Wister rat was filtered by synthetic fiber membrane and cultured under the condition of humidified 95% air admixed 5% CO₂, for 72 hours at 37℃ in the media of consisting 90% DMEM, 10% FBS and with 10ng/ml macrophage colony stimulating factor (M-CSF). Adherent cells were collected by exposure to trypsine-EDTA solution. After FBS and with 10ng/ml macrophage colony stimulating factor (M-CSF). 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 periprosthetic 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 components might later be absorbed from the circulation to the surface of the implant or implant-derived wear debris.

3) Reverse transcriptase polymerase chain reaction (RT-PCR) and PCR;
Total RNA was isolated from frozen tissues and cultured cells. Total RNA was converted into cDNA and enzymatic amplification of the specific cDNA sequences was performed on Light Cycler system (Rosch, Germany). Human and rat TLR4, 9, Tumor necrosis factor-alpha (TNF-alpha), Interleukin (IL)-1 beta, -6, and M-CSF mRNA were amplified. Quantitative analysis of the mRNAs was performed with the use of Light Cycler Software at each time. GAPDH was used as control and statistical analysis was performed by Fisher’s PLSD test.

RESULTS:
Immunohistochemistry; Conventional immunofluorescent staining showed co-localization of CD68 / TLR4 (Fig 1), CD68 / TLR9 (Fig 2) and TLR4 / TLR9 in the focal monocyte/macrophage aggregates and synovial lining both in the interface and regenerated capsular tissue samples. In addition, confocal laser system revealed that, TLR4 was expressed prominently on the cell surface, and TLR9 expression was in the cytoplasm. Expression of TLR4 and 9 in the osteoarthritic synovium was only found in vascular cells and the reactivity was weak.

mRNA analysis; In the tissues from loose hip joints, TLR4 and TLR9 were markedly detected. Monocytes/macrophages derived from rat bone marrow revealed marked expression of TLR4, 9, TNF-alpha, IL-1 beta, -6, and M-CSF. After particle stimulation, TLR4 was increased already one hour after the addition of the particles, but suppressed very soon thereafter (p < 0.05). TLR9 did not increase by one hour, but decreased after that to subnormal levels (p < 0.05) (Fig 3).

Discussion and Conclusion:
Due to the great pro-inflammatory and pro-immune potential of the TLR system, it seems interesting that expression of lipopolysaccharide receptor TLR4 and CpG DNA receptor TLR9 was so prominent in the tissues around loosening total hip replacement implants. TLR4, which recognizes not only bacterial cell lipopolysaccharide, but also self-components, heat shock proteins, hyaluronic acid, and fibrinogen, was particularly prominent on the surface of the cells, whereas TLR9, which recognizes not only CpG released during intracellular processing of microbes but also self-component, chromatin complex, was found in the cell cytoplasm. Messenger RNA levels of TLR4 and 9 were relatively constant during the initial stages of particle stimulation but decreased later after phagocytosis of the particles. This is in contrast to titanium particle induced up-regulation of osteolytic cytokines, TNF-alpha, IL-1 beta, -6, and M-CSF. This indicates that the rapidly responsive, but potent TLR system is tightly controlled via some negative feedback system so that strong and prolonged TLR-mediated stimulation does not overshoot leading to excessive and harmful host response, which might injure innocent bystander cells/tissues and might cause more harm than benefit. In conclusion, the marked expression of TLRs in macrophages in the tissues around loose hip joint and in vitro indicates high potential responsiveness of the interface membrane to microbial and endogenous TLR ligands. TLRs may contribute to various modes of loosening, such as septic loosening, particle disease and delayed type hypersensitivity reactions. TLR system itself also seems to be effectively down-regulated by implant derived wear debris, possibly to prevent exaggerated harmful inflammatory responses associated with host tissue damage.