INTRODUCTION
Aseptic loosening may occur in even a technically well-inserted prosthesis; this is the most critical problem in total hip arthroplasties (THAs). However, the mechanism of prosthetic loosening has not yet been elucidated. Histopathological analyses have indicated aseptic loosening of total hip joints is caused by foreign body associated-granulomatous reaction to wear particles, in which abundant macrophages were found in interface tissues between bone and prostheses and regenerated pseudocapsular tissues. Locally detected macrophages play a critical role in its pathogenesis after phagocytosis of the wear debris. This reaction produces various stimuli leading to osteolastogenesis and bone resorption. However, the precise mechanism responsible for signal transduction pathway has been unclear.

DGK has been recognized as a catalytic enzyme, which convert second messenger diacylglycerol (DG) to phosphatidic acid (PA) by phosphorylation. DGKs play important roles in intracellular signaling from many receptors and modulate diverse cellular processes, because of their enzyme activity influences both DG and phosphatidic acid levels. Six DGK isozymes (α, β, γ, ε, ζ, and η) have been identified in central nervous system (1). DGKα and ζ have been reported to play important role in the healing and scaring process of ischemic heart diseases (2). The aim of study was to analyze the DGK isozymes in periprosthetic aseptic loosened THAs.

METHODS
Synovial-like membranes and regenerated capsular tissues were obtained from aseptic loose total hip joints (n=7). Osteoarthritic synovium (OA) without marked inflammatory reaction was obtained at primary THR and was used as control tissues (n=7).

Quantitative mRNA analysis (RT-PCR: real-time reverse transcriptase-polymerase chain reaction)
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 (Rosche Diagnostics, Manheim, Germany). DGKα, β, γ, ε, ζ, 1 and TNF-α (an inflammatory marker) mRNA were amplified. PCR products were separated by 2% agarose gel electrophoresis. Quantitative analysis was also performed with the use of LightCycler Software (Rosche Diagnostics). β-actin, house keeping gene, was also amplified as internal control. The ratio of DGKs and TNF-α/β-actin for each samples were calculated and compared with aseptic and osteoarthritic tissues each other. Statistical analysis was performed by Fisher’s PLSD test.

Immunohistochemical analysis
Conventional immunochemical study was performed for DGKα, β, γ, ε, ζ, 1 and TNF-α (Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA). Co-localization of DGKs, CD68 (Dako Cytomation, Glostrup, Denmark: a specific marker of macrophages) and/or CD15 (Dako Cytomation: a specific marker of neutrophils), were examined by immunofluorescence staining method using Alexa fluorescent system (Molecular Probes Inc., Eugene, OR, USA).

RESULTS
Electrophoresis of PCR products
Expression of DGKα, β, γ, ε, ζ, 1 and TNF-α was detectable in all the samples from aseptic loose THAs and osteoarthritic synovium.

Quantitative mRNA analysis
Enhanced expression of DGKα, β, γ, ε, ζ, 1 and TNF-α in synovial-like membranes from aseptic loose THAs was also confirmed at mRNA levels, compared with osteoarthritic synovium. DGKζ was most dominant in synovial-like membranes from aseptic loose THAs, compared with osteoarthritic tissues (Figure 1, p<0.005).

Immunohistochemical analysis
CD68+ cells were observed in focal stromal macrophage infiltrates and synovial lining cells both in the interface and regenerated capsular tissues from aseptically loosened hip joints. DGKα, β, γ, ε, ζ, 1, and TNF-α positive cells were also seen in the macrophage infiltrates and in the synovial lining cells both in the interface and regenerated capsular tissues retrieved from aseptically loosened hip joints. Co-localization of DGKs and TNF-α with CD68 was confirmed by immunofluorescence staining (Figure 2). In osteoarthritic synovial membrane, a few scattered CD68+ cells were observed in the synovial lining and sub-lining layers and perivascularly. Expression of DGKs and TNF-α in the osteoarthritic synovium was only found in vascular cells and the reactivity was weak.

DISCUSSION AND CONCLUSION
In periprosthetic aseptic loosening/osteolysis, macrophages, which are central player of innate immunity, play a critical role of its pathogenesis. This study is a first report of expression of six DGK isozymes at mRNA level and the cellular localization of CD68+ macrophages in loose periprosthetic tissues with osteolysis. Significantly increased mRNA level of DGKs and marked immunoreactivities to DGKs in CD68+ macrophages suggests that DGK related- DG signal pathway can modulate scavenger function and inflammatory response in the process of foreign body reaction. This would be explained by the previous reports of scavenger function of macrophages with increased DGKζ level in ischemic heart diseases (2) and positive effect on the production of proinflammatory cytokines via DGK-related signal pathway in parasite infection (3, 4). Thus, DGK-related DG signal pathway seems to contribute to phagocytic host response to wear debris followed by inflammatory reaction in aseptic loose periprosthetic tissues of THAs, in which intracellular signaling on phosphorylation of DG to PA by DGKs is a key function of innate immunity and self-tolerance.