INTRODUCTION

Toll-like receptors (TLR) and CD14 expression by host innate immune cells has been proposed to be associated with the production of inflammatory cytokines, chemokines and antimicrobial molecules. TLRs expression has been found in macrophages, lymphocytes, dendritic cells, endothelial and epithelial cells as well as endothelial cells. However, LPS recognition pathways on synovial cells have not been determined. Because synovial cells are one of the major cellular components of the joint to nurture cartilage, understanding the signal transduction pathway activated by LPS on synovial cells may be of benefit in septic and non-septic arthritis therapy. We hypothesize that LPS induces inflammation in joint synovial cells through either CD14 or TLR-dependent mechanisms. In this study, we investigated the expression of TLR2/TLR4 and inflammatory cytokine productions (IL-1β, IL-6, TNF-α and IL-8) in Thy-1a/SVCs (Right panel of the figure).

METHODS

Cell Culture. Synovial specimens obtained from knee joints of 6- to 8-wk-old Tg mice SVCs were isolated using collagenase II and expanded in monolayers on plastic in DMEM growth medium. Thy-1a/CD68- SV cells were further purified using magnetic particle separation.

Antisense & LPS treatment: TLR antisense sequences were designed based on the information from the GeneBank database. The oligonucleotides were synthesized by Biognostik. Cell cultures were treated with chimeric and scrambled control oligonucleotides (10 μg/ml) for 18 hours prior LPS exposure. LPS was purchased as lyophilized, purified E. coli, Salmonella, Shigella and Pseudomonasa (Sigma, St. Louis, MO). PBS dissolved LPS were added to the cell cultures with final concentration of 10 ng/ml.

Detection of CD14/TLRs expression. RT-PCR was used to detect the message. Total cellular RNA from synovial cell cultures with or without LPS (10 ng/ml for 4 hrs) or antisense treatment were prepared using a RNA isolation kit. Primers were used as follows: TLR-2, forward primer 5’aatccagcaagctgtaaatgaacttgagtctatggtagac3’ and reverse primer 5’aacctcagacaaagcgtcaaaatc3’. For TLR-4 and TLR-6, forward primers 5’gttagtactctgatctatgtagac3’ and 5’ggtagtactctgatctatgtagac3’ respectively. Immunocytochemistry and western blotting assays were used to determine the TLR protein expression. Anti-human TLR1-6 and CD14 polyclonal antibodies (Santa Cruz Biotechnology, Inc. Santa Cruz, CA) were used to localize the positive cells and detect protein.

ELISA for cytokines and beta-Defensin in culture supernatants was performed using DuoSet kits according to the manufacturer’s instructions (R&D Systems, Minneapolis, MN). SV cells were initially cultured for 48 hours after purified through the thy1 antibody negative magnet column selection. Following this, the monolayer cell cultures were treated with either antisense or LPS. Then, supernatants were stored at –80 °C until analysis.

RESULTS

Both message and transcription levels of TLR-2, -4 and -6 were detected in isolated Thy-1a/CD68- SV cells (figure: left panel). TLR-4 was expressed at higher levels than TLR-2 was under the culture condition. Although the TLR-6 protein expression level could be detected, the expression density of both TLR-4 and TLR-6 were higher than that of TLR-6. Thy-1a/CD68- SV cells were capable responding to various gram-negative bacterial LPS exposure by production of TNF-α, IL-1β, IL-6 and B-defensin (Table of ELISA results).

The receptor expression of TLR-2, -4 and -6 could be blocked successfully using anti-TLR antisense compared with scrambled oligonucleotide controls. The cytokine and antimicrobial peptide response were inhibited significantly by anti-TLR-4 antisense treatment (Right panel of the figure).

We also found that LSP, TNF-α, IL-1β and IL-2 enhanced TLR-4 expression, but these stimuli did not increase TLR-2 expression significantly. LPS increasing cytokine and inducing beta-defensin productions was associated with NF-κB activation in Thy-1a/CD68 SV cells.

DISCUSSION

These results indicate that synovial Thy-1a/CD68 SV cells with TLR expression can respond to LPS exposure and produce inflammatory cytokines and antimicrobial peptide. LPS induced cytokine and peptide was strongly associated with TLR expressions. Anti-TLR antisense treatment significantly and specifically decreases LSP induced cytokine productions. Our data and other recent reported experimental results suggested that synovial TLR expression may be a major responsive factor for septic and reactive arthritis.