Analysis of the morphological change and the expression of secretory leukocyte protease inhibitor (SLPI) in various cell lines after lipopolysaccharide stimulation

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ABSTRACT

Bacterial lipopolysaccharide(LPS) is can stimulate the most LPS-responsive cells in the mammalian host. The macrophage response to LPS can protect the host from infection but high levels, contribute to systemic inflammatory response syndrome and destruction of host itself. The previously study, secretory leukocyte protease inhibitor (SLPI) was known LPS-induced product of macrophage and had the function that antagonizes their LPS-induced activation of pro-inflammation signaling factors. Purpose of this study was to identify the expression of SLPI involving the infection in various cell lines including odontoblast cell line. Therefore, we conducted in vitro researches, which treated the LPS to the MDPC-23, and compared to NIH3T3, RAW264.7. To investigate the expressionof SLPI in mRNA level, the methods was used RT-PCR and western blotting for protein expression of SLPI. Moreover, we performed the scanning electron microscopic (SEM) observation for the morphological change. This work was supported by Korea Science and Engineering Foundation (Project #R08-2003-000-10279-0).

INTRODUCTION

Invasion of oral bacteria commonly occurs when dentin is exposed following a breach in the overlying enamel or cementum. The products of bacteria diffuse through the dentinal tubule toward the pulp and lead to inflammatory changes in the pulpo-dentin complex. The consequence that may occur pulpitis and pulp inflammation, necrosis or infection of root canal system, periapical disease. These clinical symptoms contribute through the production of pro-inflammatory cytokines and growth factors. Secretory leukocyte protease inhibitor (SLPI) is an 11.7-kDa cystein-rich protein, as the epithelial cell product found in saliva, seminal plasma, and cervical, nasal, and bronchial mucus.

SLPI known LPS-induced product of macrophage and had the function that antagonizes their LPS-induced activation of pro-inflammation signaling factor as the primary cytokines, NF-B, nitric oxide (NO).Therefore, we have tried out determined expression of SLPI in odontoblast (MDPC-23) by infection.

MATERIAL and METHOD

MDPC-23 (odontoblast-like cells) and NIH3T3 (fibroblast), RAW 264.7 (macrophage) cells were cultured in DMEM supplemented with 10% fetal bovine serum (FBS) and 100ng/ml of E. coli LPS was added to the medium. The cells exposed to LPS for 30min, 60min and 90min, 24h, 48h, respectively and total RNA, protein extracted from cell lysates for RT-PCR, western blotting. Additionally, cell morphological change were assessed by scanning electron microscopy after fixation of the cells for 24 h in 2.5% glutaraldehyde in phosphate-buffered saline (PBS), post fixation for 1 h in OsO4, and critical point drying.

RESULT

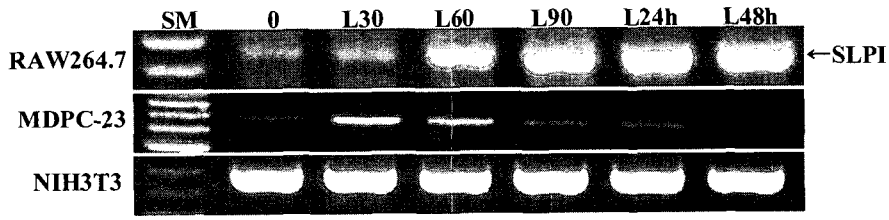

Fig. 1. SLPI mRNA expression in RAW 264.7, MDPC-23, NIH3T3cells treated with LPS for 30min, 60min, 90min, 24h, 48h, respectively. After incubation with 100ng of LPS per ml, an increase SLPI mRNA expression in RAW 264.7 cells follows at time course and its mRNA expression increase at 30min but decreased after 60min by LPS stimulation follows at time course in MDPC-23 cells. SLPI mRNA expression strongly showed in NIH3T3 cells irrelevant at time course.

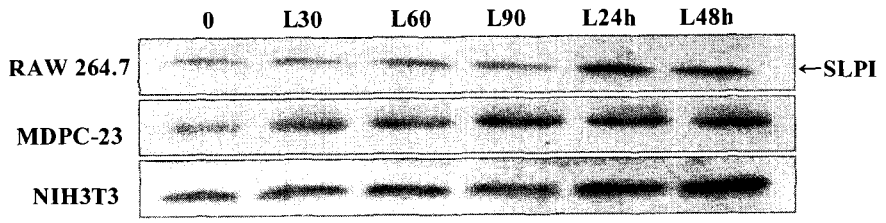

Fig. 2. Western blot analysis in RAW 264.7 cells and MDPC-23, NIH3T3 cells treated with LPS for 30min, 60min, 90min, 24h, 48h, respectively. After incubation with 100ng of LPS per ml, the SLPI protein expression was increased in RAW 264.7 cells at 24h and 48h. And SLPI protein Expression level was a whole strong aspect in NIH3T3 cells. Besides, SLPI protein expression was maintained regularly until 60min by LPS stimulation but it has decreased at 90min and 24h, 48h in MDPC-23 cells.

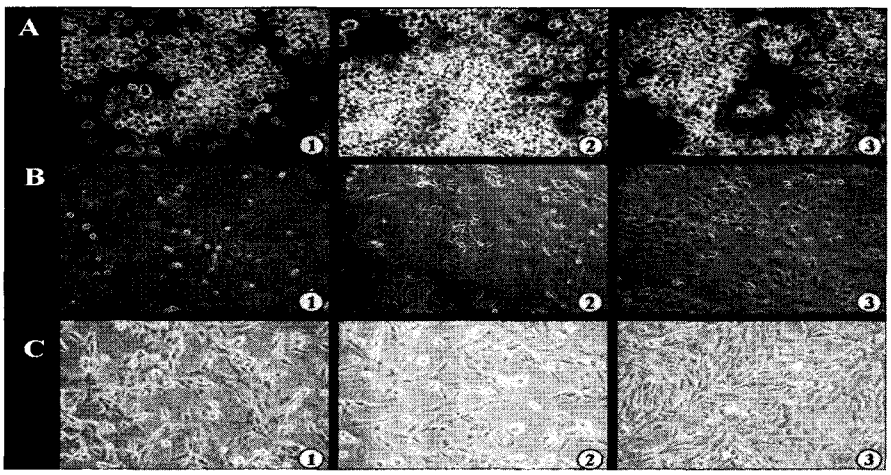

Fig. 3. RAW 264.7 (A) cells stimulation with LPS induced consistent changes in cell morphology. When at 60 min after LPS treatment, morphology alteration of RAW 264.7 cells were most excessively. MDPC-23 (B) and NIH3T3 (C) cells did not show any detectable change in morphology compared with untreated cells. The cells exposed to LPS (100ng/ml) for control (1), 30 min (2), 60 min (3), respectively. Inverted photomicrographs at x200.

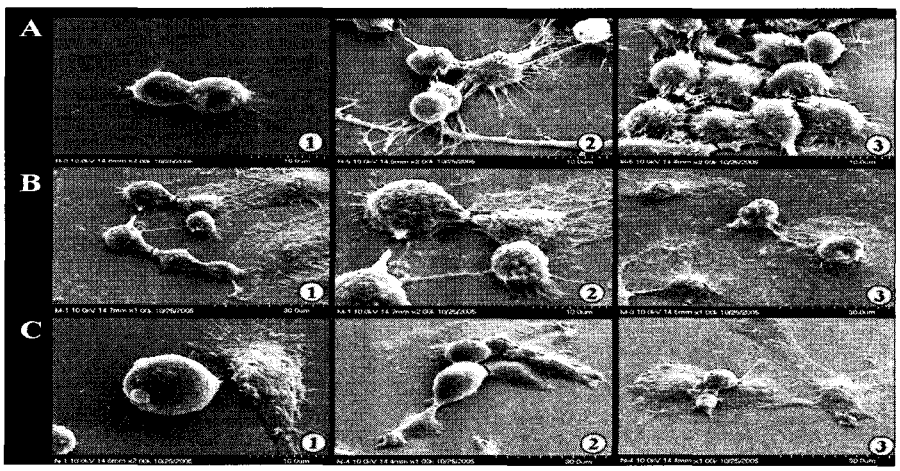

Fig. 4. Morphology of RAW264.7 (A) and MDPC-23 (B), NIH3T3 (C) cells by lipopolysaccharide using the SEM. The cells exposed to LPS (100ng/ml) for 0min30min, 60min, respectively. The cells exposed to LPS (100ng/ml) for control (1), 30 min (2), 60 min (3), respectivel