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Toll-like receptor ligands induce cytokine and chemokine production in human inner ear endolymphatic sac fibroblasts

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ABSTRACT

Objective: Against recent reports concerning cytokine or chemokine in mouse or rat inner ear cells, it is almost unknown whether human inner ear cells would produce cytokine or chemokine. We have for the first time established the human inner-ear-derived fibroblasts from endolymphatic sac.
Methods: The expression levels of Toll-like receptors (TLRs) in human endolymphatic sac fibroblasts, and the effect on cytokine or chemokine production of the TLR ligands have been examined. To demonstrate the intracellular pathways involved in the regulation of cytokine-production, we used specific inhibitors of c-Jun N-terminal kinase (JNK), extracellular signal-related kinase (ERK), p38 mitogen-activated protein kinase (p38 MAPK)-signaling and N-acetyl-L-cysteine (NAC).
Results: TLR 2, 3, 4 and 9 were highly expressed in human endolymphatic sac fibroblasts. The TLR 3 ligand, polyinosinic–polycytidylic acid (poly(I:C)) significantly enhanced the secretion of thymic stromal lymphopoietin (TSLP), B lymphocyte stimulator (BLyS), IFNγ-inducible protein 10 (IP-10), and macrophage inflammatory protein 1 alpha (MIP-1α) from the cells. The inhibitor of JNK strongly reduced the poly(I:C)-induced TSLP-production. The antioxidant drug, NAC also reduced the TSLP-production in fibroblasts stimulated with poly(I:C).
Conclusion: Our findings suggest human inner-ear-endolymphatic sac derived fibroblasts can produce the cytokine and chemokine in response to TLR ligands and play a certain role during the initiation of an immune response.

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1. Introduction

Recently there have been several reports concerning cytokine or chemokine in mouse or rat inner ear cells [1–4]. It is still little known whether human inner ear cells would produce cytokine or chemokine. The endolymphatic sac is capable of antigen recognition and processing for initiation of an immune response, and potent molecules directly toxic to invading pathogens are expressed by the sac epithelium. The keyhole limpet hemocyanin (KLH) challenge of the scala tympani induced more inflammatory cells expressing the proinflammatory cytokines in the perisaccular tissue of the endolymphatic sac contained [5]. The microarrays have revealed that multiple key elements of both the cellular and humoral innate immune system including Toll-like receptors (TLRs) were expressed in human endolymphatic-sac tissue samples [6].

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There has been not any report which TLR ligand can induce its production cytokine or chemokine in human inner ear cells. Recently we have established fibroblast lines from the human endolymphatic sac. Since fibroblasts, a rich source of cytokines and chemokines, play a key role in the innate response, here we have examined the expression levels of TLRs in human endolymphatic sac fibroblasts, and the effect on cytokine or chemokine production of the TLR ligands including poly-inosinic–polycytidylic acid (poly(I:C)), and lipopolysaccharide (LPS). In order to demonstrate the intracellular pathways involved in the regulation of cytokine-production, we used specific inhibitors of c-Jun N-terminal kinase (JNK), extracellular signal-related kinase (ERK), p38 mitogen-activated protein kinase (p38 MAPK)-signaling and N-acetyl-L-cysteine (NAC) that is one of the known antioxidants on the event induced by double-stranded RNA (dsRNA).

2. Materials and methods

2.1. Reagents

The following reagents were used: poly(I:C) (Amersham Bioscience, Piscataway, NJ, USA), LPS (MERCK Bioscience, Germany), NAC (Santa Cruz, Texas), SP600125 as a specific inhibitor of JNK (BIOMOL), SB203580 as a specific inhibitor of p38 MAPK (Promega), PD98059 as a specific inhibitor of MEK-1 (Promega), anti-phosphorylated JNK (SAPK/JNK) Ab (Cell Signaling), and anti-JNK (SAPK/JNK) Ab (Millipore).

2.2. Cells, cell lines, and cell culture

Human fibroblast lines were established from biopsy tissues ($n = 6$) of endolymphatic sac removed during the operation, and they were characterized as previously described [7]. The fibroblasts were cultured in RPMI-1640 medium supplemented with 10% fetus calf serum and in a humidified atmosphere of 10% CO_2 in air at 37 °C. The microphotographs of cultivated fibroblasts are shown in Fig. 1.

All the endolymphatic sac specimens had been obtained from patients with intractable Meniere's disease that was designated in cases where various forms of medical and psychological management failed for more than six months. The patients received a clinical diagnosis of Meniere's disease according to the 1995 AAO-HNS criteria [8]. The endolymphatic sac surgery procedure involved a simple mastoidectomy that clearly exposed the endolymphatic sac in the area between the sigmoid sinus and the inferior margin of the posterior semicircular canal as described in [9]. The sac was opened with an L-shaped incision, and then the edge of incised sac was partially removed as biopsy tissue. This study was performed according to Declaration Helsinki. The subjects had given written informed consent, and the study protocol was approved by the Ethics Committee of University of Fukui.

2.3. Real-time PCR

Total RNA was extracted using a total RNA isolation NucleoSpin™ RNA II Kit (MACHERY-NAGEL, Düren, Germany). The reverse transcription reaction was performed with TaqMan® RT Reagents (Applied Biosystems Japan, Tokyo, Japan) using random hexamer primers. The amplification of TLRs and β_2-microglobulin-cDNA was performed in a MicroAmp optical 96-well reaction plate (Applied Biosystems). All TaqMan® probe/primer combinations used in this study were TaqMan® Gene Expression Assay products purchased from Applied Biosystems. β_2-Microglobulin was chosen as the reference housekeeping gene because it is convenient to assay and highly expressed. Furthermore, in order to select a housekeeping gene, we conducted evaluation using a TaqMan® Human Endogenous Control Plate, which was the most suitable. TaqMan® PCR was performed in a 20-μl volume using the TaqMan® Universal PCR master mix (Applied Biosystems). The reaction was performed in an ABI PRISM 7000 Sequence Detection System (Applied Biosystems). Reaction mixtures were pre-incubated for 2 min at 50 °C. The PCR program was 10 min of Taq Gold activation at 95 °C, followed by 40 cycles of 15 s at 95 °C and 1 min at 60 °C (maximum ramping speed between temperatures). Human cDNA equivalent to 50 ng of total RNA from each sample was assayed in each tube. The threshold cycle number was determined with Sequence Detector System software (version 1.1: Applied Biosystems) and transformed using comparative methods as described by the manufacturer, with β_2-microglobulin as the calibrator gene.

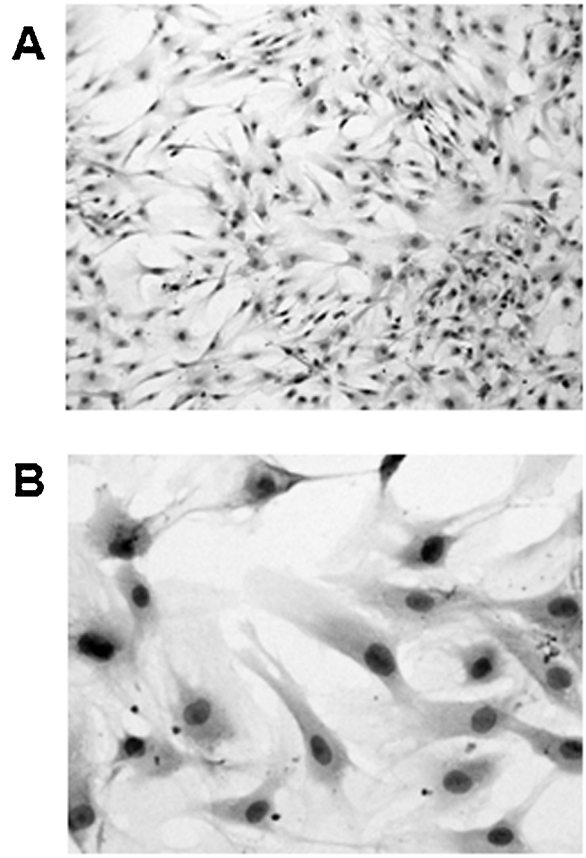

Fig. 1. Microphotographs of human endolymphatic sac fibroblasts. After purified fibroblasts were seeded and cultured on chamber slide-glasses. The section was stained hematoxylin–eosin and examined with light microscope. (A) 100×, (B) 400× magnification.

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