Polyinosinic-Polycytidylic Acid Induces the Expression of GRO-α in BEAS-2B Cells

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Abstract—Growth-related oncogene protein-α (GRO-α)/CXCL1 is a chemokine that activates neutrophils and plays an important role in inflammatory reactions. Polyinosinic-polycytidylic acid (poly IC) is a synthetic double-stranded RNA (dsRNA), which is a ligand for Toll-like receptor-3. Poly IC mimics viral infection when applied to cells and induces inflammatory and immune responses. In the present study, we found the induction of GRO-α in BEAS-2B bronchial epithelial cells treated with poly IC. Pretreatment of cells with 2-aminopurine, an inhibitor for dsRNA-dependent protein kinase (PKR), inhibited the expression of GRO-α induced by poly IC. Overexpression of interferon-regulatory factor-3 (IRF-3) or retinoic-acid inducible gene-I (RIG-I) enhanced the induction of GRO-α by poly IC. PKR, IRF-3, and RIG-I may be involved in the poly IC-induced expression of GRO-α in BEAS-2B cells. Airway viral infection may elicit GRO-α expression in the bronchial epithelium, which may be implicated in inflammatory and immune reactions.

Key Words: poly IC; bronchial epithelial cells; GRO-α; dsRNA; IRF-3; RIG-I.

INTRODUCTION

Double-stranded RNA (dsRNA) is generated during RNA virus infection and induces antiviral responses in host cells. Polyinosinic-polycytidylic acid (poly IC) is a synthetic dsRNA and elicits various cellular responses similar to those provoked by viral infection.

Bronchial epithelial cells serve as one of the important components of immune and inflammatory reactions against viral infection by expressing various functional molecules including cytokines and adhesion molecules. These reactions contribute not only to host defense mechanisms but also to acute and chronic respiratory diseases. It has been reported that bronchial epithelial cells express Toll-like receptor-3 (TLR-3) that recognizes dsRNA (1). Treatment of bronchial epithelial cells with poly IC induces the expression of various cytokines including interleukin-8 (IL-8), IL-6, RANTES (regulated on activation, normal T cells expressed and secreted), granulocyte-macrophage colony-stimulating factor (GM-CSF), and monocyte chemoattractant protein-1 (MCP-1) (2–5).

IL-8/CXCL8 and growth-related oncogene-α (GRO-α)/CXCL1 are members of CXC chemokine family, and they promote the chemotaxis and activation of neutrophilic leukocytes. These chemokines are produced by bronchial epithelial cells upon stimulation and enhance inflammatory reactions in the airway (6). Expression of
IL-8 induced by poly IC or virus has been well characterized (2–4). However, there is no information about the expression of GRO-α in bronchial epithelial cells during antiviral responses. The present study was undertaken to address the expression of GRO-α in BEAS-2B bronchial epithelial cells in response to poly IC.

MATERIALS AND METHODS

Reagents

Dulbecco’s modified Eagle medium (DMEM), fetal bovine serum (FBS), primer oligo (dT)12–18, and M-Mulv reverse transcriptase were purchased from InVitrogen (Carslbad, CA, USA). Poly IC and 2-aminopurine (2-AP) were from Sigma (St. Louis, MO, USA). Cycloheximide (CHX) was from Wako (Osaka, Japan). An RNeasy RNA isolation kit, Taq DNA polymerase and an Effectene transfection reagent were from Qiagen (Hilden, Germany). A Quantikine enzyme-linked immunosorbent assay (ELISA) kit for GRO-α was obtained from R & D Systems (Minneapolis, MN, USA). Oligonucleotide primers were synthesized by Greiner Japan (Atsugi).

Cells Cultures

BEAS-2B cells, a cell line derived from human bronchial epithelial cells (7), were cultured using DMEM supplemented with 10% FBS (8). The cells were stimulated with poly IC (∼20 µg/mL) for up to 24 h. In the experiments using 2-AP or CHX, the cells were pretreated for 30 min with one of these reagents and then stimulated with poly IC.

RNA Extraction and Reverse Transcription-Polymerase Chain Reaction (RT-PCR)

RT-PCR analysis of GRO-α expression was performed as reported previously (9). Briefly, total RNA was isolated from the cells, and single-strand cDNA was synthesized from 1 µg of total RNA using a primer oligo (dT)12–18 and the M-Mulv reverse transcriptase. Specific primers were designed from cDNA sequences of GRO-α, IL-8, and glyceraldehyde-3-phosphate dehydrogenase (GAPDH); and each cDNA was amplified by PCR using Taq DNA polymerase. The sequences of the primers were as follows:

GRO-α-F (5’-ATGGCCCGCGCTGCTCTCC-3’),
GRO-α-R (5’-AGTGGTGTTGCTAAGAATTCCA-3’),
IL-8-F (5’-GGATGCTAAGAAGAATCTCC-3’),
IL-8-R (5’-GGCTGGAGTCTAAGAATTCCA-3’),
GAPDH-F (5’-CCACCCATGGCAAATCTCCATGGCA-3’), and
GAPDH-R (5’-TCTAGACGGCAGGTCCAGGTCCACC-3’).

The conditions for reactions were 1 × (94°C, 1 min), 30 × (94°C, 1 min; 55°C, 1 min; 72°C, 1 min), and 1 × (72°C, 10 min). The products were analyzed by electrophoresis on a 1.8% agarose gel containing ethidium bromide. The expected size of the PCR products for GRO-α, IL-8, and GAPDH was 321, 219, and 598 bp, respectively. Because all of these primer pairs were designed from different exons, the products with the expected size were amplified from single-strand cDNA but not from the contaminating genomic DNA. The PCR products were confirmed to be specific for GRO-α, IL-8, and GAPDH by sequencing.

ELISA for GRO-α

The level of GRO-α protein in the conditioned medium of BEAS-2B cells was measured using an ELISA kit. The conditioned medium was collected and subjected to centrifugation at 10,000 × g for 10 min, and the supernatant was used as samples for ELISA.

Transfection of Cells with RIG-I or IRF-3 cDNA

The cDNA for interferon responsible factor-3 (IRF-3) or retinoic-acid inducible factor-I (RIG-I) was transfected into BEAS-2B cells using an Effectene transfection reagent according to the supplier’s protocol. After 24 h of incubation with the transfection mixture, the cells were washed twice and fresh medium was added. Then the cells were stimulated with 10 µg/mL poly IC for the indicated period of time. When the cells were transfected with the control construct encoding green fluorescence protein (GFP), GFP was detected in about 70% of the cells after 24 h of transfection incubation (not shown).

RESULTS

Expression of GRO-α mRNA and Protein in BEAS-2B Cells Stimulated With Poly IC

A small amount of GRO-α mRNA was detected, by RT-PCR, in unstimulated BEAS-2B cells. Treatment of cells with poly IC enhanced the expression of GRO-α mRNA and protein in a concentration-dependent manner (Fig. 1A and B). The time course of GRO-α expression in BEAS-2B cells treated with poly IC is shown in Fig. 2.