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Inverse relationship between *Sec14l3* mRNA/protein expression and allergic airway inflammation

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ABSTRACT

Bronchial asthma is an inflammatory disease of the airways. The *Sec14l3* gene, encoding a 45-kDa secretory protein, is specifically expressed in airway epithelium. Here, we report on the kinetics of *Sec14l3* expression following allergic inflammation of the lung. Brown Norway rats were sensitized by intraperitoneal injection of ovalbumin, followed by challenge with aerosolized ovalbumin after a 3-week interval. This animal model showed many features similar to human allergic asthma: an increase in inflammatory cells such as eosinophils, lymphocytes and neutrophils in bronchoalveolar lavage (BAL) fluid and histopathological alteration of lung tissue, exhibiting infiltration of these inflammatory cells and degeneration and necrosis of alveolar epithelium. These parameters reached their maximal level 24 h after allergen challenge. In contrast, quantitative polymerase chain reaction analyses demonstrated a rapid and significant reduction of *Sec14l3* mRNA in lung tissue and maximum reduction (to 1.4% of the control) was observed at 24 h. Pretreatment with dexamethasone significantly suppressed both the *Sec14l3* mRNA reduction and all of the inflammatory changes. The 45-kDa secretory protein was identified in the supernatant of BAL fluids. Two-dimensional gel images of the supernatant proteome also revealed down-regulation of the protein following inflammation (to approximately 30% of the control at 24 h). Thus, *Sec14l3* expression is highly and inversely associated with the progression of airway inflammation. *Sec14l3* mRNA and protein may function in the homeostasis of airway epithelial cells under normal conditions.

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1. Introduction

Bronchial asthma is an inflammatory disease of the airways characterized by many diagnostic observations, including chronic inflammation, eosinophilic infiltration, reversible airway obstruction, late asthmatic response and airway hyperresponsiveness (Barnes, 1996; Bousquet et al., 1990; Roche et al., 1989). These characteristics suggest that immunological changes and inflammatory cell activation by allergen invasion may provide important indicators that would be useful as diagnostic and therapeutic targets for asthma.

The airway epithelium plays a critical role in airway defenses by protecting the respiratory tract from infection and damage induced by inhaled toxins, pathogens and particles. Airway epithelial cells also have a much wider range of immunological activities including the release of eicosanoids, endopeptidases, cytokines and chemokines that are important in the pathogenesis of allergic inflammation (Bellini et al., 1993; Marini et al., 1992; Pawankar, 2002). During the inflammatory process, the epithelium is injured, and then repaired and regenerated to restore its function (Coraux et al., 2005). Therefore, impairment of airway epithelium is also a key function in the process of inflammatory airway diseases.

Various proteins are secreted by a number of airway epithelial cells ranging from olfactory to alveolar cells (Ghafouri et al., 2004; Hermans and Bernard, 1999). One of these proteins, the Sec14-like protein 3 (*Sec14l3*), was originally isolated and cloned as a water-soluble 45-kDa secretory protein from rat olfactory epithelium (Merkulova et al., 1999; Novoselov et al., 1996). This protein belongs to the family of mammalian Sec14-like proteins, which is composed of at least three paralogs (Sec14l2, 3 and 4). These proteins are thought to mediate the intracellular transport of hydrophobic ligands and/or ligand-specific signal transduction (Kempna et al., 2003). Recently, it was shown that the *Sec14l3*/45-kDa protein accumulates in extracellular vesicles together with phosphatidylinositol-3,4,5-triphosphosphate (PIP3), a highly phosphorylated inositol phospholipid (Merkulova et al., 2005).

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Rat *Sec14l3* mRNA expression has been detected in the apical region of the trachea and in the surface layer of ciliated bronchial epithelium in the lung (Merkulova et al., 1999). In the present study, we report that *Sec14l3* expression is inversely regulated with airway inflammation in a mammalian asthma model. Moreover, we also found quantitative differences in the gene-encoded 45-kDa protein in bronchoalveolar lavage (BAL) fluids after allergen challenge. These results suggest that *Sec14l3* may provide a quantitative monitor of the progression of airway inflammatory diseases such as asthma. Possible molecular mechanisms mediating *Sec14l3* inverse regulation are discussed.

2. Materials and methods

2.1. Animals

Male Brown Norway (BN) rats of 6 weeks of age were purchased from Seac Yoshitomi Ltd. (Fukuoka, Japan). Animals were housed under specific pathogen-free conditions and freely provided with laboratory food and water. All animal experiments were performed in accordance with The Ethical Guidelines for Animal Experiments by Tsukuba Research Laboratories of GlaxoSmithKline K.K.

2.2. Sensitization and allergen challenge

Animals were sensitized by intraperitoneal injection of ovalbumin (1 mg/rat, Sigma-Aldrich Inc., St Louis, MO, USA) together with $Al(OH)_3$ (alum, 100 mg/rat; Wako Pure Chemical Industries, Ltd., Osaka, Japan) in 0.9% saline for 3 consecutive days. Three weeks after the third ovalbumin–alum injection, the animals were exposed to aerosolized 3% w/v ovalbumin in 0.9% saline delivered with an ultrasonic nebulizer (NE-U12, OMRON Co., Tokyo, Japan) for 15 min. Animals in the control group were exposed to aerosolized 0.9% saline.

2.3. BAL and cell analysis

Animals were killed by exsanguination under anesthesia with pentobarbital (50 mg/kg, Dainippon Sumitomo Pharma Co., Osaka, Japan) at 1, 3, 6, 24, 48, 72 and 96 h after allergen challenge. BAL was then performed with 10 ml (2 ml×5) of phosphate-buffered saline (PBS) warmed to 37 °C. The recovered BAL fluid was centrifuged (500×*g*, 10 min at 4 °C), and the pellet of cells was suspended with 0.5 ml of PBS. The total number of cells in the BAL fluid was counted with a microcell-counter (F-800, Sysmex Co., Kobe, Japan). Cytospin slides were prepared from the cell suspension and stained with Diff-Quik (Sysmex International Reagents Co., Kobe, Japan). Differential cell counting of at least 300 cells was performed according to standard morphologic criteria by light microscopy.

2.4. Histopathological examination

Histopathological study of the lungs was performed to determine lung histology during airway inflammation in our model system. The left lobes of the lungs were dissected from the individuals different from those for BAL procedures. The lobes were then fixed in 10% neutral buffered formalin for 24 h at room temperature. The fixed tissues were routinely embedded in paraffin and then sectioned and stained with hematoxylin and eosin (H&E). H&E samples were then examined under a light microscope. Histological findings were scored according to the following criteria: [0] = no abnormality detected, [1] = minimal, [2] = mild, [3] = moderate and [4] = marked changes. The intensity and extent of airway inflammation were compared with the control group by one blinded observer in the low (×40) to high (×400) power fields using light microscopy (S. A.).

2.5. RNA extraction and real-time quantitative reverse transcriptase-polymerase chain reaction (RT-PCR)

Dissected tissues were rapidly frozen in liquid nitrogen, and stored at −80 °C until use. Total RNA was isolated from the tissues using an RNeasy Mini Kit (QIAGEN GmbH, Hilden, Germany) according to the manufacturer's instructions. *Sec14l3* mRNA expression in rat tissues was quantified by an ABI PRISM® 7700 sequence detection system (Applied Biosystems Inc., Carlsbad, CA, USA) (Heid et al., 1996). We designed a pair of PCR primers to amplify a portion of the *Sec14l3* mRNA and a fluorogenic TaqMan hybridization probe (Applied Biosystems). The sequences of the PCR primers were: 5′-CTGGCCAAGTTCCGAGAAAAT-3′ (forward primer) and 5′-TCGGAGCCAACGAAGAAGA-3′ (reverse primer). The TaqMan probe (5′-Fam-CGCCCTGCCAAACCCTGATGACTATT-Tamura-3′) hybridized to the template in between the two PCR primers. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as an endogenous control to normalize each sample. The GAPDH primers and probe were purchased from Applied Biosystems (TaqMan Rodent GAPDH Control Reagents). Each real-time RT-PCR mixture contained Master Mix (at a 1× final concentration), 6.25 U of a MultiScribe reverse transcriptase, 10 U of ribonuclease inhibitor (Applied Biosystems), 300 nM of the forward primer, 900 nM of the reverse primer, 200 nM of the TaqMan probe and 45 ng of total RNA in a total volume of 25 µl. Amplification was performed in duplicate. The real-time RT-PCR reactions were started with an initial reverse transcription step at 48 °C for 30 min. After denaturation at 95 °C for 10 min the RT-PCR was carried out by performing 40 cycles of incubation at 60 °C for 1.5 min followed by incubation at 95 °C for 15 s.

2.6. Drug administration

Dexamethasone 21-phosphate disodium salt was obtained from Sigma-Aldrich, and was dissolved in 0.9% saline. The solution was intraperitoneally administered at 2 and 24 h before the allergen challenge.

2.7. Protein separation and identification

Proteins in both the supernatant and precipitate (cell pellet) fractions of the BAL fluid (Section 2.3), taken from animals 24 h after allergen challenge, were separated by two-dimensional electrophoresis (2-DE). For analysis of the BAL fluid supernatant trichloroacetic acid solution was added to a 1-ml aliquot of the supernatant to a final concentration of 10% w/v. After standing at 4 °C for 1 h, the protein aggregate was collected by centrifugation, washed with cold diethylether and dried under vacuum. The resulting dried pellet was dissolved with a 2-DE sample buffer (Kawakami et al., 2000). For analysis of the BAL fluid precipitate, sample buffer was directly added to the precipitate from one half of the fluid collected from one individual. 2-DE and protein identification were carried out as described previously (Kawakami et al., 2000) with minor modifications. Briefly, the sample solution was mixed with standard proteins (Kamo et al., 1995) and subjected to 2-DE. After electrophoresis, protein spots on the 2-DE gel were stained with the SYPRO® Ruby fluorescent dye (Invitrogen Co., Carlsbad, CA, USA), and spot images were acquired with a laser scanner. After spot detection, the isoelectric point (p*I*) and mass value of each sample protein spot were measured and corrected with the standard protein spots. Sample protein spots were excised from the 2-DE gel plate and subjected to in-gel tryptic hydrolysis, followed by peptide extraction (Shevchenko et al., 1996). The resulting peptide mixture was analyzed by reversed phase liquid chromatography, directly coupled with electrospray ionization-tandem mass spectrometry (Aebersold and Mann, 2003) using an LCQ ion trap mass spectrometer (Thermo Fisher Scientific Inc., Waltham, MA, USA). The tandem mass spectrometry data were used to search for corresponding sequences in amino acid sequence

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