Upregulation of geranylgeranyltransferase I in bronchial smooth muscle of mouse experimental asthma: its inhibition by lovastatin

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Abstract

RhoA has been recognized as an important protein for bronchial smooth muscle (BSM) contraction and hyperresponsiveness, and its activation is also regulated by geranylgeranyltransferase I (GGTase I). In the present study, the effects of repeated antigen exposure on the expression of GGTase I were determined in mouse BSMs. Male BALB/c mice were sensitized and repeatedly challenged with ovalbumin antigen. Animals were also treated with lovastatin (4 mg/kg/day, i.p.) once a day prior to and during the antigen inhalation period. Western blot analyses revealed that GGTase I was upregulated in BSMs of the antigen-challenged mice. The systemic treatment with lovastatin attenuated the upregulation of GGTase I induced by antigen exposure. Interestingly, lovastatin also significantly reduced the protein expression of GGTase I in BSMs of control animals. We thus concluded that an upregulation of GGTase I in BSM might be, at least in part, involved in the development of antigen-induced airway hyperresponsiveness. Lovastatin might have therapeutic potential to ameliorate airway hyperresponsiveness in allergic bronchial asthma.

Key words: geranylgeranyltransferase, lovastatin, asthma, bronchial smooth muscle, hyperresponsiveness

Introduction

The dramatic increase in the number of asthma cases over the last decades is of great concern for public health in the world (Eder et al., 2006). Increased airway narrowing in response to nonspecific stimuli is a characteristic feature of human obstructive diseases, including bronchial asthma. This abnormality is an important sign of the disease, although the pathophysiological variations leading to the hyperresponsiveness are unclear now. It has been suggested that one of the factors that contribute to the exaggerated airway narrowing in asthmatics is an abnormality of the properties of airway smooth muscle (Martin et al., 2000; Seow et al., 1998). Rapid relief from airway limitation in asthmatic patients by b-stimulant
inhalation may also suggest an involvement of augmented airway smooth muscle contraction in the airway obstruction. Thus, it may be important for development of asthma therapy to understand changes in the contractile signaling of airway smooth muscle cells associated with the disease.

There is increasing evidence that a monomeric GTP-binding protein, RhoA, and its downstream target, Rho-kinases, are involved in the Ca\(^{2+}\)-independent contraction (termed Ca\(^{2+}\) sensitization) of airway smooth muscles (Chiba \textit{et al.}, 1999; 2005; Ito \textit{et al.}, 2001; Yoshii \textit{et al.}, 1999). When the RhoA/Rho-kinase system is activated by contractile agonists, the activity of myosin light chain (MLC) phosphatase is reduced and the level of phosphorylated MLC is then increased, resulting in an augmentation of contraction. Recent studies demonstrated that the agonist-induced, RhoA/Rho-kinase-mediated Ca\(^{2+}\) sensitization of bronchial smooth muscle (BSM) contraction is augmented in rats (Chiba \textit{et al.}, 1999) and mice (Chiba \textit{et al.}, 2005) with allergic bronchial asthma. An importance of the RhoA/Rho-kinase system has also been demonstrated in human BSM (Yoshii \textit{et al.}, 1999), and the signaling of RhoA and its downstream Rho-kinases are now considered as a therapeutic target for the treatment of airway hyperresponsiveness in asthma (Gosens \textit{et al.}, 2006; Kume, 2008; Schaafsma \textit{et al.}, 2008a; 2008b).

It is known that posttranslational geranylgeranylation of RhoA protein is required for its activation and/or membrane translocation (Somlyo and Somlyo, 2003; Takai \textit{et al.}, 2001). Indeed, statins, which indirectly inhibit posttranslational prenylation by depleting the 3-hydroxy-3-methyl-glutaryl-CoA (HMG-CoA) downstream metabolites farnesylpyrophosphate and geranylgeranylprophosphate, can inhibit translocation of RhoA to plasma membrane, resulting in an inhibition of RhoA-mediated function in cultured tumor cell lines (Lee \textit{et al.}, 2006; Zhong \textit{et al.}, 2003). Our previous studies also revealed that lovastatin can ameliorate BSM hyperresponsiveness by reducing the membrane-associated RhoA in animal models of allergic bronchial asthma (Chiba \textit{et al.}, 2008a; 2008b). More recently, it has also been suggested that the level of geranylgeranylated RhoA is increased in BSM of mouse experimental asthma (Chiba \textit{et al.}, 2009). The result reminds us of an increased activity of geranylgeranyltransferase (GGTase) in BSM of the disease. So in the present study, the expression level of GGTase I, a main GGTase that contributes to RhoA geranylgeranylation (Yokoyama \textit{et al.}, 1991; Yokoyama and Gelb, 1993), in BSM tissues of antigen-induced airway hyperresponsive mice was compared with that of control animals. The effects of systemic treatment with lovastatin on the expression levels of GGTase I in BSMs were also determined.

**Methods**

\textit{Animals and treatments}

Male BALB/c mice were purchased from the Charles River Japan, Inc. (Kanagawa, Japan) and housed in a pathogen-free facility. All animal experiments were approved by the Animal Care Committee of Hoshi University (Tokyo, Japan).

Preparation of a murine model of allergic bronchial asthma was performed as described previously (Chiba \textit{et al.}, 2005; 2008b; 2009). In brief, mice (8 weeks of age) were actively
sensitized by intraperitoneal injections of 8 µg ovalbumin (OA; Seikagaku Co., Tokyo, Japan) with 2 mg Imject Alum (Pierce Biotechnology, Inc., Rockfold, IL, USA) on days 0 and 5. The sensitized mice were challenged with aerosolized OA-saline solution (5 mg/ml) for 30 min on Days 12, 16 and 20. A control group of mice received the same immunization procedure but inhaled saline aerosol instead of OA challenge. In another series of experiments, animals also received intraperitoneal injection with lovastatin (Toronto Research Chemicals, Inc., Ontario, Canada; 4 mg/kg/day; dissolved in 50% DMSO in PBS) or its vehicle once a day for 10 days from Day 11 to Day 20 as previously described (Chiba et al., 2009). The treatment was carried out 1 hour before the antigen inhalation in case of the days of the antigen challenge (Days 12, 16 and 20). Twenty-four hour after the last OA challenge, mice were sacrificed by exsanguination from abdominal aorta under urethane (1.6 g/kg, i.p.; Sigma-Aldrich, St. Louis, MO, USA) anesthesia, and protein samples of BSM tissues were prepared as previously described (Chiba et al., 2005; 2008b; 2009).

**Western blot analyses**

Protein samples were subjected to 15% SDS-PAGE and the proteins were then electrophoretically transferred to a PVDF membrane. After blocking with 3% gelatin, the PVDF membrane was incubated with polyclonal rabbit anti-GGTase I antibody (1 : 1,000 dilution; Abgent, San Diego, CA, USA). Then the membrane was incubated with horseradish peroxidase-conjugated donkey anti-rabbit IgG (1 : 2,500 dilution; Amersham Biosciences, Co., Piscataway, NJ, USA), detected by an enhanced chemiluminescent system (Amersham Biosciences, Co.) and analyzed by a densitometry system. Detection of house-keeping gene was also performed on the same membrane by using monoclonal mouse anti-GAPDH (1 : 10,000 dilution; Chemicon International, Inc., Temecula, CA, USA) and horseradish peroxidase-conjugated sheep anti-mouse IgG (1 : 2,500 dilution; Amersham Biosciences, Co.) to confirm the same amount of proteins loaded.

**Statistical analysis**

All the data are expressed as the mean ± S.E. Statistical significance of difference was determined by analysis of variance (ANOVA) with post hoc Bonferroni/Dunn (StatView for Macintosh ver. 5.0, SAS Institute, Inc., NC, USA). A value of $P<0.05$ was considered significant.

**Results**

Figure 1 shows the effect of repeated antigen exposure on the expression level of GGTase I protein in BSMs of mice. Immunoblot analysis using the anti-GGTase I antibody showed a band at 44 kDa, the molecular weight corresponding to GGTase I protein (Fig. 1, upper panel). As compared to the nonsensitized normal control animals, the protein expression of GGTase I was significantly increased in BSMs of the repeatedly antigen-challenged mice ($P<0.01$; Fig. 1, lower panel).

The effects of systemic treatment with lovastatin on the protein expression of GGTase I were also determined in BSMs of control and repeatedly antigen challenged mice. As shown in
Fig. 2, the systemic treatment with lovastatin significantly reduced the protein levels of GGTase I not only in the antigen-challenged mice but also the control ones.

**Discussion**

Using our well-established mouse model of allergic bronchial asthma (Chiba et al., 2005; 2008b; 2009), the effect of repeated antigen exposure on the expression level of GGTase I was determined. As a result, we show here for the first time that GGTase I is upregulated in BSMs of the antigen-challenged mice (Fig. 1). An inhibitory effect of lovastatin on the expression of GGTase I in BSMs was also suggested (Fig. 2).

It has been suggested that one of the factors that contribute to the airway
hyperresponsiveness in asthmatics is an abnormality of the nature of airway smooth muscle (Martin et al., 2000; Seow et al., 1998). In the mouse model of allergic bronchial asthma prepared by the same procedure currently used, an increased responsiveness of the isolated BSM to acetylcholine was observed (Chiba et al., 2005; 2008b; 2009). An augmented agonist-induced, RhoA-mediated Ca\(^{2+}\) sensitization of BSM contraction accompanied by an upregulation of RhoA protein has also been demonstrated (Chiba et al., 2005). In addition to the upregulation of RhoA, the current study revealed that the expression level of GGTase I, a main GGTase that contributes to RhoA geranylgeranylation (Yokoyama et al., 1991; Yokoyama and Gelb, 1993), was increased in the diseased BSMs (Fig. 1). It is known that posttranslational geranylgeranylation of RhoA protein is required for its activation (Somlyo and Somlyo, 2003; Takai et al., 2001). It is thus possible that the RhoA-mediated signaling in BSMs of the disease might be extremely enhanced both by the increments of RhoA protein itself and its

![Fig. 2. Effects of systemic treatments with lovastatin on the antigen-induced upregulation of geranylgeranyltransferase I (GGTase I) protein in bronchial smooth muscle tissues of mice. The GGTase I protein levels in bronchial smooth muscles of control and repeatedly antigen-challenged animals (Challenged) were determined by Western blot analyses. Lovastatin (4 mg/kg/day; filled columns) or its vehicle (50% DMSO in PBS; open columns) was also pretreated intraperitoneally for 10 days as described in METHODS. (Upper) Typical photographs of immunoblots for GGTase I (44 kDa) and GAPDH (36 kDa). The density ratios of the GGTase I to the GAPDH bands were calculated and the levels of GGTase I protein expressions were expressed as % of the vehicle-treated control, and the data are summarized in the lower panel. Each column represents the mean ± S.E. from 5–6 different animals. * P<0.05 vs. respective vehicle-treated group by Bonferroni/Dunn test.](image)
geranylgeranylation. Indeed, our recent study revealed that the level of geranylgeranylated RhoA is increased in BSM of mouse experimental asthma (Chiba et al., 2009).

Statins, such as lovastatin, could inhibit GGTase activity by depleting its substrate geranylgeranylpyrophosphate, a downstream metabolite of HMG-CoA. So we expected that the protein expression of GGTase I might be upregulated by the systemic treatment with lovastatin for keeping the homeostasis of certain functional geranylgeranylated proteins. Surprisingly, the current study revealed that the systemic treatment with lovastatin significantly reduced the expression of GGTase I in BSM tissues of both control and antigen-challenged animals (Fig. 2). Because statins are known to have pleiotropic effects beyond lowering cholesterol levels, one of the possible actions of lovastatin may be regulation of GGTase I expression in the mouse BSM. Further studies are required to make clear the exact mechanism of downregulation of GGTase I by lovastatin.

Because the prevalence of asthma is recently rising (Umetsu et al., 2002), there is an increased need for the development of new drugs for its treatment, especially for patients who respond poorly to conventional therapy such as glucocorticoids. Recently, statins have been proposed as a novel treatment of respiratory diseases including asthma (Hothersall et al., 2008; Imamura et al., 2009; Takizawa, 2007; Walsh, 2008). Although their effectiveness to asthma is still controversial (Hothersall et al., 2008; Menzies et al., 2007), statins could improve the airway hyperresponsiveness by inhibiting GGTases indirectly (Chiba et al., 2008a; 2008b). Recently, it has also been suggested that systemic treatment with GGTI-2133, an inhibitor of GGTase I, attenuated the BSM hyperresponsiveness induced by antigen exposure (Chiba et al., 2009). The current study revealed that the protein expression of GGTase I was increased in the BSM of mouse experimental asthma (Fig. 1). It is thus possible that selective inhibition of GGTases is effective for asthma therapy by improving the airway hyperresponsiveness.

In conclusion, the present study clearly demonstrates that GGTase I is upregulated in BSMs of the antigen-challenged mice. Lovastatin has an ability to reduce the expression of GGTase I in BSM, although its mechanism of action is unclear now.

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References


GGTase expression in asthma


