

Increased expression of G α q protein in bronchial smooth muscle of mice with allergic bronchial asthma

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Abstract

In the present study, the change in Gq protein level in bronchial smooth muscle of mice with antigen-induced airway hyperresponsiveness (AHR) was determined. BALB/c mice were actively sensitized and repeatedly challenged with ovalbumin antigen to induce bronchial smooth muscle hyperresponsiveness. The contraction induced by 10 μ M AlF₄⁻ (generated by 10 μ M AlCl₃ plus 10 mM NaF) of bronchial smooth muscles isolated from the antigen-challenged mice was significantly augmented as compared with that from the control animals. The G α q protein level determined by immunoblotting was also significantly increased in bronchial smooth muscles of the antigen-challenged group. Thus, an upregulation of G α q protein may be involved in the pathogenesis of bronchial smooth muscle hyperresponsiveness, one of the causes of AHR in asthmatics.

Key words: asthma, airway hyperresponsiveness, bronchial smooth muscle, heterotrimeric GTP-binding protein, Gq

Introduction

Asthmatics have an increased vagal tone and hyperresponsiveness to various stimuli such as muscarinic receptor agonists *in vivo*. Bronchial smooth muscles isolated from patients with the *in vivo* airway hyperresponsiveness (AHR) also have an augmented contractility to methacholine (Roberts *et al.*, 1984). Similar observations have also been reported in animal models of asthma (Chiba and Misawa, 1993; Misawa and Chiba, 1993; Rubinfeld *et al.*, 1982). It is thus possible that the change in bronchial smooth muscle itself might be involved in the AHR, at least in part.

Using saturation and competition radioligand binding techniques, we have previously demonstrated that the muscarinic receptor affinity to acetylcholine is markedly increased in airways of an asthma model, although the expression level of the receptors was not changed (Chiba and Misawa, 1995). Interestingly, the enhanced affinity returned to the control level in

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the presence of guanosine 5'-O-(3-thiotriphosphate) (GTP γ S) (Chiba and Misawa, 1995), suggesting that levels of heterotrimeric GTP-binding proteins (G proteins) linked with the muscarinic receptors might be increased in airways of asthmatics. The Gq protein is known to be linked to the muscarinic M₃ receptor that is undoubtedly the main receptor subtype contributing to airway smooth muscle contraction (Barnes, 1990). The current study was therefore carried out to determine whether or not the Gq protein level in bronchial smooth muscle is increased at the AHR using a mouse model of allergic bronchial asthma.

Methods

Sensitization and antigenic challenge

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 *et al.*, 2004; Chiba *et al.*, 2005; Chiba *et al.*, 2006). In brief, mice (8 weeks of age) were actively sensitized by intraperitoneal injections of 8 μ g ovalbumin (OA; Seikagaku Co., Tokyo, Japan) with 2 mg Imject Alum (Pierce Biotechnology, Inc., Rockford, IL, USA) on day 0 and day 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. Twenty-four hours after the last OA challenge, mice were sacrificed by exsanguination from abdominal aorta under urethane (1.6 g/kg, *i.p.*; Sigma, St. Louis, MO, USA) anesthesia.

Functional study

The contraction of the mouse bronchial tissue was measured as described previously (Chiba *et al.*, 2004). In brief, about 3 mm length of the left main bronchus (about 0.5 mm diameter) was isolated and epithelium was removed. The resultant tissue ring preparation was then suspended in a 5 mL-organ bath containing oxygenated Krebs-Henseleit solution (118.0 mM NaCl, 4.7 mM KCl, 2.5 mM CaCl₂, 1.2 mM MgSO₄, 25.0 mM NaHCO₃, 1.2 mM KH₂PO₄, 10.0 mM glucose, pH 7.4). After an equilibration period, contractile response induced by 10 μ M AlF₄⁻ was measured in the presence of atropine (10⁻⁶ M), indomethacin (10⁻⁶ M) and propranolol (10⁻⁵ M). AlF₄⁻ (10 μ M) was generated as follows; the bronchial rings were pretreated with AlCl₃ (10 μ M) for 5 min, and then given an addition of NaF (10 mM).

Western blot

Protein samples of the bronchial smooth muscle tissue were prepared as described previously (Chiba *et al.*, 1999b; Chiba *et al.*, 2005). Samples were subjected to 12% SDS-PAGE and the proteins were then electrophoretically transferred to a PVDF membrane. After blocking, the PVDF membrane was incubated with the polyclonal rabbit anti-G α q antibody (1:1,000 dilution; Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA). Then the membrane was incubated with horseradish peroxidase-conjugated donkey anti-rabbit IgG (1:2,500 dilution; Amersham

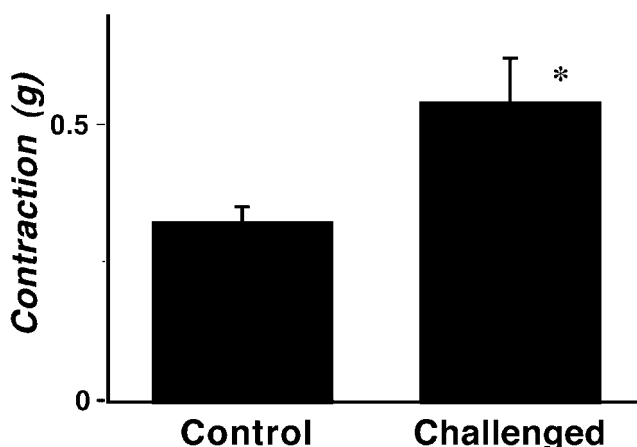


Fig. 1. AIF₄⁻-induced contractile responses of bronchial rings isolated from sensitized control (Control) and repeatedly antigen challenged mice (Challenged). AIF₄⁻ (10 μ M) was generated in a 5-mL organ bath as follows; the bronchial rings were pretreated with AlCl₃ (10 μ M) for 5 min and then NaF (10 mM) was added. Each column represents the mean $\pm$ S.E.M. from 6 different animals. The AIF₄⁻-induced contraction is significantly augmented in the bronchial smooth muscle of the antigen-challenged mice as compared to that of control animals ($P < 0.05$).

Biosciences, Co., Piscataway, NJ, USA), detected by an enhanced chemiluminescent system (Amersham Biosciences, Co., Piscataway, NJ, USA) and analyzed by a densitometry system. Detection of house-keeping gene was also performed on the same membrane by using monoclonal mouse anti-GAPDH antibody (1:3,000 dilution; Chemicon International, Inc., Temecula, CA, USA).

Statistical analyses

All the data are expressed as the mean $\pm$ standard error of mean (S.E.M.). Statistical significance of difference was determined by unpaired Student's *t*-test. Probabilities of less than 5% ($P < 0.05$) were considered significant.

Results and discussion

Using our well-established mouse model of allergic bronchial asthma (Chiba *et al.*, 2004; Chiba *et al.*, 2005; Chiba *et al.*, 2006), which has a marked AHR (Chiba *et al.*, 2004), the effect of repeated antigen exposure on the expression of a heterotrimeric GTP-binding protein, Gq, in bronchial smooth muscle was determined.

Figure 1 shows the contractile responses of mouse bronchial smooth muscles induced by 10 μ M AIF₄⁻ that was generated by adding 10 mM NaF 5 min after treatment with 10 μ M AlCl₃. In each tissue, AIF₄⁻ induced a delayed and gradual contraction, and the contractile response reached a plateau 15–20 min after the addition of NaF. AIF₄⁻ is able to activate G proteins, including Gq, directly in intact tissues and cells (Hall, 1992; Li *et al.*, 1995). AIF₄⁻ induces the dissociation of heterotrimeric G proteins into the α monomer and $\beta\gamma$ dimer in a GTP-independent manner (Bokoch *et al.*, 1983; Howlett *et al.*, 1979). The ability of AIF₄⁻ to activate

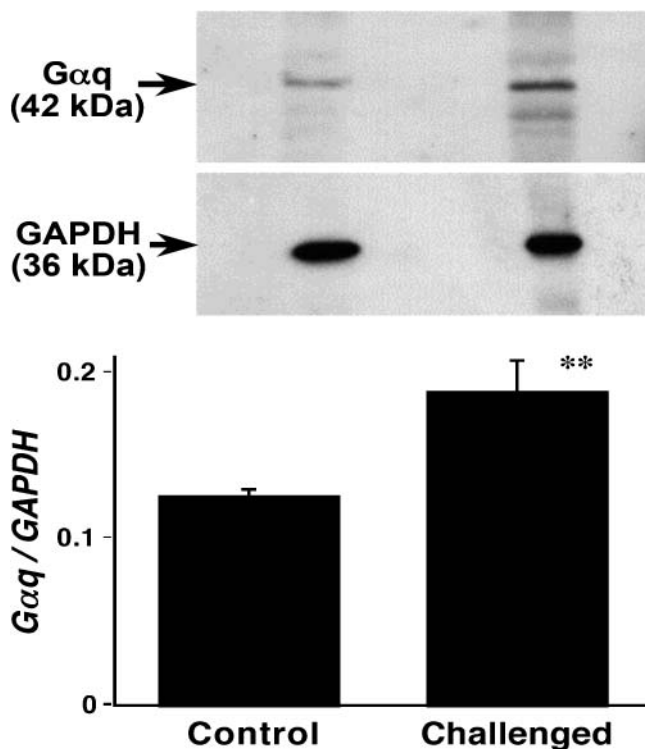


Fig. 2. The level of the expression of Gαq protein in the bronchial smooth muscle tissues from sensitized control (Control) and repeatedly antigen-challenged mice (Challenged). *Upper panel:* Typical photographs of immunoblots for Gαq (42 kDa) and GAPDH. The bands were scanned and the level of Gαq protein expression was expressed as density ratio of the Gαq to the GAPDH bands, and the data are summarized in the *lower panel*. Each column represents the mean $\pm$ S.E.M. from 6 different animals. The expression of the Gαq protein is significantly increased in the bronchial smooth muscle of the antigen-challenged mice as compared to that of control animals ($P < 0.01$).

G proteins is due to its ability to mimic the γ -phosphate of GTP at the GTP-binding site on the G protein (Bigay *et al.*, 1985). Similarly, AlF_4^- is also known to induce a significant increase in inositol phosphate accumulation in tracheal smooth muscle cells (Yang *et al.*, 1994b). It is thus possible that the contraction induced by AlF_4^- might be mainly mediated by the generation of inositol phosphate probably via the activation of Gq proteins.

In the mouse allergic bronchial asthma currently used, the bronchial smooth muscle contraction induced by contractile agonists such as acetylcholine and endothelin-1, but not by non-receptor stimuli such as high K^+ depolarization, was markedly augmented (Chiba *et al.*, 2004). Currently, the maximal contractile response observed in the repeatedly antigen challenged mice was significantly greater than that in the sensitized control animals ($P < 0.05$; Fig. 1). This finding indicates that enhanced signaling has occurred at the level of Gq protein and/or its downstream pathway(s) in the augmented bronchial smooth muscle contractility. Indeed, the level of Gαq protein in bronchial smooth muscle was significantly increased in the repeatedly antigen

challenged mice ($P < 0.01$; Fig. 2). In agreement with the present study, an augmented AIF₄-induced contraction with an upregulation of Gαq protein was also reported in bronchial smooth muscle of a rat model of antigen-induced AHR (Chiba *et al.*, 2000). Moreover, Lee *et al.* (1994) reported an increased level of Gαq/11 protein in lungs from chronically OA-challenged guinea pigs that have AHR to acetylcholine, although they did not distinguish Gαq and Gα11. The over-expression of Gαq protein may thus be a universal event of the pathogenesis of AHR over species.

Muscarinic stimulation induces an increase in the GTPase activity, which is sensitive to the selective muscarinic M₃ receptor antagonist 4-diphenylacetoxy N-methylpiperidine (4-DAMP) and anti-Gαq antibody in airways (Lee *et al.*, 1994), indicating the enhanced coupling of muscarinic M₃ receptors to Gq proteins. It is therefore suggested that the enhanced contractile response via muscarinic M₃ receptors in AHR may reflect the over-expression of Gq proteins in airway smooth muscles. As well as muscarinic M₃ receptors, many other receptors participate in airway smooth muscle contraction, such as histamine H₁ (Daykin *et al.*, 1993), serotonin 5-HT₂ (Yang *et al.*, 1994c), leukotrienes (Dumitriu *et al.*, 1997), tachykinin NK₂ (Grandordy *et al.*, 1988), bradykinin B₂ (Yang *et al.*, 1994a) and endothelin ET (Hersch *et al.*, 2004), are known to couple to phospholipase C via the Gq proteins. In addition, recent studies also revealed that the Gαq directly activates a monomeric GTP-binding protein, RhoA, via a formation of Gαq-p63RhoGEF-RhoA complex (Lutz *et al.*, 2005; 2007). RhoA is involved in the agonist-induced Ca²⁺ sensitization of smooth muscle contraction, and an augmentation of the RhoA-mediated Ca²⁺ sensitization in the bronchial smooth muscle hyperresponsiveness has been demonstrated (Chiba *et al.*, 1999; Chiba *et al.*, 2005). Thus, an attractive possibility is that the 'nonspecific' AHR might be due to over-expressed Gq proteins in airway smooth muscles.

In conclusion, an increased expression of Gαq protein in bronchial smooth muscle may be involved in the augmented contraction and the pathogenesis of AHR.

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