Suppression of prostaglandin E2-induced MUC5AC overproduction by RGS4 in the airway

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1The Airway Mucus Institute, 2Department of Otorhinolaryngology, 3Brain Korea 21 for Medical Sciences, and 4Research Center for Human Natural Defense System, Yonsei University College of Medicine, Seoul; and 5Department of Anatomy, College of Medicine, Yonnam University, Taegu, Korea

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Song KS, Choi YH, Kim JM, Lee H, Lee TJ, Yoon JH. Suppression of prostaglandin E2-induced MUC5AC overproduction by RGS4 in the airway. Am J Physiol Lung Cell Mol Physiol 296: L684–L692, 2009. First published February 6, 2009; doi:10.1152/ajplung.90396.2008.—The mechanism by which E-prostanoid (EP) receptor is critically involved in PGE2-induced mucin 5AC (MUC5AC) gene expression in the airway has been unclear. Furthermore, there have been little reports on the negative regulatory mechanisms that affect stimulant-induced MUC5AC gene expression in the airway.

PGE2, one of the major prostaglandins produced, is a versatile eicosanoid that regulates key responses in numerous physiological and pathological processes, including angiogenesis, tumorigenesis, immune modulation, and inflammatory airway diseases (7, 33). PGE2 exerts its biological functions through E-prostanoid (EP) cell surface receptors. EP1,4 receptors couple to Gαq, Gαs, Gαi, and Gαo, respectively, and can activate or inhibit several signaling proteins resulting in the increased formation of secondary messengers. Recently, Gray et al. (14) reported EP2 and EP4 may be the major receptors for IL-1β-induced MUC5AC gene expression by producing PGE2 in the normal human tracheobronchial epithelial cells, and Kim et al. (20) suggested that EP4 is essential for PGE2-induced MUC5AC secretion in normal human nasal epithelial (NHNE) cells. However, the exact mechanism by which EP receptor is critically involved in PGE2-induced MUC5AC gene expression has been unclear. Furthermore, we wanted to find a negative regulator of PGE2-induced MUC5AC gene expression at the EP receptor level.

We thought a member of regulators of G protein signaling (RGS) proteins may play as a negative regulator of PGE2-induced MUC5AC gene expression since RGS proteins can stimulate the G protein GTPase activity and thereby produce desensitization (deactivation). RGS proteins are defined by a shared 120-amino acid domain that binds directly to activated Gα subunits (3) and act as tightly regulated modulators and integrators of G protein signaling. A protein domain, known as the RGS box, harbors GTPase-accelerating proteins (GAP) activity and is the defining feature of RGS proteins. Outside the RGS box, the presence of other protein domains diversifies the range of protein interactions and regulatory activities of RGS proteins. Binding of RGS proteins to active Gα (GTP-bound) can interfere with effector-binding proteins, thereby blocking activation and downstream signaling (16). In addition, the expression of RGS2, -3, -5, and -16 subtypes, mainly members of R4 RGS subfamily, has been detected in the lung (22, 26). In the airway, which RGS protein has a critical role and how the interplay between EP receptor and RGS leads to the alteration of MUC5AC overproduction remain poorly understood.

MUCINS are heavily glycosylated, high molecular weight glycoproteins produced by the epithelia of the respiratory, gastrointestinal, and reproductive tracts (25, 35). Twenty-one mucin genes have been identified; however, it has been unclear how mucins are regulated by various stimulants and are secreted in respiratory diseases (6). Of these, mucin 5AC (MUC5AC) is one of the major mucin genes in the human respiratory tract, and MUC5AC mRNA levels are known to be upregulated by proinflammatory cytokines, LPS, neutrophil elastase, cigarette smoking, or reactive oxygen species (15, 19, 31, 32, 36) via various signaling pathways. Nonetheless, there have been few reports on the negative regulatory mechanisms that affect stimulant-induced MUC5AC gene expression in the airway.

In the present study, we examined the mechanism by which PGE2 increases MUC5AC overproduction at the receptor level. We show that the EP4 receptor plays an important role in PGE2-induced MUC5AC overproduction in the airway. In addition, RGS4 suppressed PGE2-induced MUC5AC overproduction both in vitro and in vivo by binding to EP4-coupled Goαi depending on GTPγS.

MATERIALS AND METHODS

Materials. All chemical compounds were purchased from Cayman Chemical (Ann Arbor, MI). Go, and Gβ and Gδ antibodies were purchased from Calbiochem (Merck; Darmstadt, Germany), RGS4 and actin antibodies were purchased from Santa Cruz Biotechnology (Santa Cruz, CA), and MUC5AC antibody was purchased from Lab Vision (Fremont, CA). The cDNA expression constructs encoding EPα, Goα, Goγ, Q227L, RGS2, RGS3, RGS4, and RGS5 were purchased from the UMR cDNA Resource Center (Rolla, MO). All of small interfering RNAs (siRNAs) were synthesized from Bioneer (Daejeon, Korea): EPα, 5'-UUlCUCUCCAGGCAUGUAU (dTdT)-3'; RGS4, 5'-GGAUCCACUGUGAGAGAGUA (dTdT)-3'; and negative control, 5'-CCUACGCACACUUCUGCU (dTdT)-3'.

Cell cultures. NHNE cells (2 × 10⁵ cells/well) were seeded in 0.5 ml of culture medium on Transwell clear culture inserts (24.5 mm, 0.45-μm pore size; Costar, Cambridge, MA). Cells were cultured in a 1:1 mixture of BEMG and DMEM containing all the supplements previously described (39). Human tissue was maintained in accordance with the guidelines and approval of the institutional review board of Yonsei University, Seoul, Korea. Consent from both patients and the local ethics committee was obtained for use of the specimens. The human lung mucopidermoid carcinoma cell line (NCI-H292) was purchased from the American Type Culture Collection (CRL-1848; Manassas, VA) and cultured in RPMI 1640 (Invitrogen; Carlsbad, CA) supplemented with 10% fetal bovine serum in the presence of penicillin-streptomycin at 37°C in a humidified chamber with 5% CO₂.

Cell transfection. Cells were plated in 6-well plates 1 day before transfection with plasmid DNA (1 μg/well) or siRNA (100 pmol/well) using FuGENE 6 (Roche; Indianapolis, IN) according to the manufacturer's instructions. Approximately 24 h after transfection, cells were maintained in 0.2% serum RPMI media for 16–18 h before treatment with PGE2, and then harvested 24 h after treatment.

Measurement of cAMP level. Cells were plated in 6-well plates 1 day before transfection. Approximately 24 h after transfection, transfected cells were replated at 2,000/well in 96-well plates and maintained in 0.2% serum RPMI media for 16–18 h before treatment with PGE2. cAMP production was measured according to the manufacturer's instructions (Promega; Madison, WI).

PCR. After reverse transcription, RT-PCR was performed to amplify DNA fragments. All of EP primers (18) were synthesized from Bioneer. PCR products were run for 35 cycles using the amplification conditions (18). Real-time PCR was performed using a Bio-Rad iCycler iQ Detection System (Bio-Rad, Hercules, CA) with iQ SYBR Green Supermix. The following primers were used: human MUC5AC, forward 5'-CACGCCCCGCTCCAATGTA-3' and reverse 5'-ACCGCATTTGGGTCATCC-3'; RGS4, forward 5'-GAGAGATCTGGGCTACATGGAGGAGC-3' and reverse 5'-TTAGCTGGGAAAGGCCCACCA-3'; mouse Muc5ac primers, forward 5'-CCATGCGAGTCTCAGGACAAATCTGGC-3' and reverse 5'-TTACGTGGAAAGGGCCACCA-3'; and mouse GADPH primers, forward 5'-TGGTCGGTGTGCCGCTGATCGA-3' and reverse 5'-GCCGCTTCACCCAACCACCTGATG-3' (34). Parameters were 95°C for 10 min, followed by 40 cycles of 95°C for 15 s and 60°C for 60 s. The result was normalized against β2-microglobulin as an internal control (mouse GADPH for Muc5ac mRNA), and the relative quantity of MUC5AC mRNA was determined using a MUC5AC standard curve.

Western blot analysis, dot blot analysis, and immunoprecipitation. These methods were described previously (32). NCI-H292 cells were grown to confluence in 6-well plates. After treatment with PGE2, the cells were lysed with 2% lysis buffer [250 mM Tris·HCl (pH 6.5), 2% SDS, 4% 2-mercaptoethanol, 0.02% Bromphenol blue, 10% glyco]. Equal amounts of whole cell lysates from protein samples were resolved by 10% SDS-PAGE and transferred to a polyvinylidene difluoride membrane (PVDF; Millipore, Bedford, MA). For dot blot analysis, 125 μl of protein lysates were directly diluted with dot blot buffer (600 mM KSCN, 10 mM EDTA, pH 8.0) in 96-well plates. After serial dilution, lysates were applied by vacuum using a dot blot apparatus. The membrane were incubated with MUC5AC antibody as primary antibody and then incubated with anti-mouse hors eradish peroxidase conjugated IgG. The signal was detected by enhanced chemiluminescence kit, and the relative fold increase was calculated by dividing stimulant-treated signal into control signal since there is no commercially available MUC5AC protein for standardization. The data were represented as means ± SD of triplicate cultures from the independent experiments. For immunoprecipitation, cells were transfected with either wild-type Goαi, or dominant negative Goi, or targeted neo primer (5'-CACATGGACACTGGTGCCCTTTGC-3'), which produced a 226-bp and a 484-bp band from the wild-type and the targeted allele, respectively. For intra-tracheal instillation, the trachea was surgically exposed after anesthetization by making an incision in the neck skin. A microsyringe carrying a 31-gauge needle filled with PGE2 solution was used for injection into the exposed trachea. PGE2 (50 μl; 0.5, 1.0, or 5.0 mg/kg) was injected into the lumen of each trachea. After injection, the skin was sutured. At 1 day after injection, the mouse was killed, and the trachea was removed (34).

RESULTS

PGE2-induced MUC5AC gene expression was mediated via the EP4 receptor in NHNE cells. To determine whether PGE2 increases MUC5AC gene expression in NHNE cells, real-time...
PCR analysis was performed. As seen in Fig. 1A, PGE₂ induced MUC5AC gene expression in a dose-dependent manner with an EC₅₀ value of 73.31 ± 3.13 nM. Since Kunikata et al. (21) reported that endogenous PGE₂ played an important feedback function in allergic inflammation, and we thought that newly synthesized endogenous prostaglandins by exogenous PGE₂ might influence the results, we wanted to rule out this effect. Accordingly, indomethacin, a nonselective cyclooxygenase-1 (COX-1) and COX-2 inhibitor, was employed to investigate the function of exogenous PGE₂. Cells were treated for 24 h with PGE₂ alone or pretreated indomethacin (3 µM) for 1 h before PGE₂ treatment. Indomethacin alone had no effect, and pretreatment of indomethacin did not affect PGE₂-induced MUC5AC gene expression (Fig. 1B). This result suggests that MUC5AC gene expression have been affected by exogenous PGE₂ but not endogenous PGE₂ in the present study. We next examined whether mRNA level of EP receptors was affected by PGE₂. All of EP receptors were expressed, and treatment with PGE₂ did not affect EP receptor gene expression in NHNE cells (Fig. 1C). To identify which EP receptors are involved in PGE₂-mediated MUC5AC gene expression, cells were treated for 24 h with the EP₁<sub>3α</sub>-selective agonist, sulprostone, or the EP₂<sub>α</sub>-selective agonist, misoprostol (Fig. 1D; Ref. 14). Misoprostol (10 µM) increased MUC5AC mRNA level, whereas 10 µM sulprostone had no effect. This result indicates that EP₂ and/or EP₄ were (was) involved in PGE₂-induced MUC5AC gene expression. Moreover, to identify which EP receptor has a substantial role in MUC5AC gene expression, a nonselective EP₂ antagonist, AH6809 (20, 30), and a selective EP₄ antagonist, GW627368X (37, 38), were used (Fig. 1E). The increase in MUC5AC gene expression by PGE₂ treatment (7.33-fold) is decreased by ~42.1% on addition of AH6809 (4.25-fold), whereas GW627368X inhibited ~73.6% (1.94-fold) of it. This result suggests that both EP₂ and EP₄ receptors are probably related to PGE₂-induced MUC5AC gene expression in NHNE cells. However, in our previous study, EP₄ receptor played as a major receptor for PGE₂-induced MUC5AC production at the transcriptional and translational levels (20). Accordingly, we selected the EP₄ receptor as a major receptor in the present study. To complement this pharmacological approach, we used the genetic strategy of siRNA in NCI-H292 cells. As shown in Fig. 1F, siRNA EP₂ and siRNA EP₄ suppressed intracellular EP₂ and EP₄ mRNA level in NCI-H292 cells, respectively. Consistent with the pharmacological study, the increase in MUC5AC gene expression by PGE₂ treatment (7.19-fold) was decreased by ~53.0% on addition of siRNA EP₂ (3.42-fold), whereas siRNA EP₄ inhibited ~71.3% (2.09-fold) of increased MUC5AC gene expression. These results suggest that both EP₂ and EP₄ receptors are probably related to PGE₂-induced MUC5AC gene expression. However, the EP₄ receptor served as a major receptor in PGE₂-induced MUC5AC gene expression.

PGE₂-mediated Gα<sub>o</sub> activity was dependent on the EP₄ receptor. Next, to identify which EP receptor has a critical role in PGE₂ signaling, the ligand selectivity of PGE₂ was examined using cAMP production assay. NCI-H292 cells were transiently transfectected with EP₂, EP₄, or mock construct. PGE₂-induced cAMP production was higher in cells transfected with EP₄ construct compared with cells transfected with EP₂ construct (Fig. 2A). The cAMP concentration (685.1 ± 14.9 pM) of the EC₅₀ value of EP₄-mediated cAMP production was much higher than that of EP₂ (462.33 ± 23.79 pM) (Fig. 2B), suggesting that EP₄ has higher sensitivity to PGE₂ compared with EP₂. It also indicated that that PGE₂ increases Gα<sub>o</sub> activity in a dose-dependent manner and that the EP₄ receptor appears to have more functional activity than the EP₂ receptor, at least in part in NCI-H292 cells. To verify that the concentration of cAMP increased by PGE₂ was mediated by EP₄, siRNA EP₄ was employed. Whereas PGE₂-induced cAMP production was higher in cells transfected with wild-type EP₄ construct compared with cells treated with PGE₂ alone, PGE₂-induced cAMP production was significantly suppressed by siRNA EP₄ construct in a dose-dependent manner (Fig. 2C). These results indicate that PGE₂-mediated Gα<sub>o</sub> activity was dependent on the EP₄ receptor.

RGS4 could regulate PGE₂-induced MUC5AC gene expression by interaction with Gα<sub>o</sub> in a GTPγS-dependent manner. The main aim of this study is to identify specific molecule(s) that suppresses PGE₂-induced MUC5AC overproduction during airway mucosal inflammation. We therefore screened inhibitory G protein molecules that attenuate G protein signaling. We checked the inhibitory effects of RGS proteins containing GTPase activity on MUC5AC gene expression. It has been reported that mainly R4 RGS family proteins (RGS2, -3, -5, and -16), containing only RGS domain, are expressed in the lung (22, 26). Of these RGS proteins, because RGS16 has been upregulated in germinal center and activated T cells, suggesting that it has a role in adaptive immunity (8), we have excluded RGS16 from this study, and selected RGS2, -3, -4, and -5 proteins. Since these RGS subtype constructs have been tagged with hemagglutinin (HA), Western blot analysis was performed with anti-HA antibody. No change was detected in the expression of HA in cells transfected with each RGS construct (Fig. 3A, top). Of the RGS proteins tested, only ectopic RGS4 expression inhibited PGE₂-induced MUC5AC gene expression (Fig. 3B, bottom). To determine a potential role for RGS4 in PGE₂-mediated MUC5AC gene expression, siRNA RGS4 was employed. Whereas wild-type RGS4 dramatically suppressed PGE₂-induced MUC5AC gene expression, siRNA RGS4 much increased PGE₂-induced MUC5AC gene expression compared with PGE₂ alone (Fig. 3B). This phenomenon is due to the inhibition of endogenous RGS4 expression. In addition, to investigate a potential role for the RGS4 in Gα<sub>o</sub>-mediated signal processing, we asked whether RGS4 bound to Gα<sub>o</sub> as a component of the G protein complex.

RGS4 plays as a negative regulator molecule for PGE₂-dependent Muc5ac gene expression in vivo. To determine the in vivo relevance of in vitro results, approximately 6- to 8-wk-old male C57BL/6 mice were tested. The trachea of mice...
Fig. 1. PGE\textsubscript{2} induces MUC5AC gene expression via the E-prostanoid (EP) receptor 4 (EP\textsubscript{4}) in normal human nasal epithelial (NHNE) and human lung mucoepidermoid carcinoma (NCI-H292) cells. 

\textbf{A}: NHNE cells were treated for 24 h with several concentrations of PGE\textsubscript{2}. Cell lysates were harvested for real-time quantitative PCR. Corresponding dissociation curves for standard (0.01 fg to 1 ng; \textit{Aa}) and samples (\textit{Ab}) were amplified using specific primers with SYBR Green dye. These curves were merged on the same panel (\textit{Ac}).

\textbf{B}: NHNE cells were treated for 1 h with 3 μM indomethacin (indo), a cyclooxygenase-1 (COX-1) and COX-2 inhibitor, before treatment for 24 h with 100 nM PGE\textsubscript{2}. Cell lysates were harvested for real-time quantitative PCR. *P < 0.05 compared with control.

\textbf{C}: NHNE cells were treated with 100 nM PGE\textsubscript{2} for 24 h. The presence of EP receptors mRNA was detected by RT-PCR with specific EP receptors primers, respectively.

\textbf{D}: NHNE cells were treated for 24 h with 100 nM PGE\textsubscript{2}, 10 μM EP\textsubscript{1/3} agonist, sulprostone, or 10 μM EP\textsubscript{2/4} agonist, misoprostol. Cell lysates were performed real-time quantitative PCR. *P < 0.05 compared with control.

\textbf{E}: NHNE cells were treated for 1 h with either 10 or 50 μM EP\textsubscript{2} antagonist, AH6809, or 10 or 50 μM EP\textsubscript{4} antagonist, GW627368X, before treatment for 24 h with 100 nM PGE\textsubscript{2}. Cell lysates were harvested for real-time quantitative PCR. *P < 0.05 compared with PGE\textsubscript{2} alone. F: NCI-H292 cells transfected with construct encoding wild-type EP\textsubscript{2} or EP\textsubscript{4}, small interfering RNA (siRNA) EP\textsubscript{2} or EP\textsubscript{4}, or siRNA control were treated for 24 h with 100 nM PGE\textsubscript{2}. Cell lysates were harvested for PCR. *P < 0.05 compared with PGE\textsubscript{2} alone and **P < 0.05 compared with overexpressed EP\textsubscript{2} or EP\textsubscript{4}. All data shown are representative of 3 independent experiments. Tm, melting temperature; β\textsubscript{2}M, β\textsubscript{2}-microglobulin; WT, wild-type; siEP2, siRNA EP\textsubscript{2}; sicon, siRNA control.
was surgically exposed under general anesthesia and aseptic conditions, and 50 μl of 0.1, 1.0, and 5.0 mg/kg PGE2 was intratracheally instilled with a 31-gauge needle. After 24 h, PCR and dot blotting for Muc5ac expression were performed using the trachea from the killed mice (Fig. 4, A and B). PGE2 increased MUC5AC expression in vivo at both the transcriptional and translational levels. These results were consistent with the in vitro results. In addition, to investigate whether RGS4 has an effect on PGE2-induced Muc5ac expression in vivo, Rgs4 KO mice were employed. After Rgs4tm1Dgen/tm1Dgen mice were genotyped by PCR with genomic tail DNA using specific primers, which produced a 226-bp and a 484-bp band from the wild-type and the targeted allele, respectively (data not shown), tracheotomy was performed for PGE2 instillation. Twenty-four hours later, total RNA and tissue lysates were purified from the trachea of Rgs4 KO mice. PGE2-induced Muc5ac expression was much increased in Rgs4 KO mice compared with wild-type mice at both the transcriptional and translational levels (Fig. 4, C and D), indicating that RGS4 probably has a suppression effect on PGE2-induced Muc5ac overproduction in the airway. When lentivirus (Fig. 4E, top), PGE2 instillation to the trachea of Rgs4 KO mice that had been infected with lenti::Mock-eGFP (Fig. 4E, bottom), indicating that administration of RGS4 to Rgs4 KO mice airway restores its suppressive function in Rgs4 KO mice. These results were consistent with in vitro results (Fig. 3B), and an inhibitory effect of RGS4 in wild-type mice. Next, to investigate whether EP4 is responsible for PGE2-induced Muc5ac overproduction, we injected wild-type mice with the EP4-specific agonist, PGE1-OH, and expression level of Muc5ac mRNA was determined. After the mice were preinjected with 1.0, 2.5, and 5.0 mg/kg GW627368X (ip) for 1 h, PGE1-OH or PGE2 was instilled intratracheally. The EP4 agonist increased Muc5ac mRNA level, whereas the mRNA level strongly inhibited GW627368X in a dose-dependent manner, and PGE2-induced mRNA level was also inhibited by GW627368X (Fig. 4F), indicating that EP4 receptor is essential for PGE2-induced Muc5ac overproduction. These results suggest that PGE2 binds to the EP4 receptor thereby inducing MUC5AC overproduction, and RGS4 may be a key negative regulator during airway mucosal inflammation.
DISCUSSION

PGE₂ is frequently observed at high concentrations at the sites of inflammation and plays physiological roles in the regulation of mucosal immunity (9). Secreted PGE₂ stimulates epithelial cells in an autocrine and paracrine fashion. Epithelial cells thereby increase mucin secretion and mucin gene expression, including MUC5AC and MUC8 (20). Interestingly, even though the importance of PGE₂ in human airway epithelium is well-established (29), the molecular mechanism by which PGE₂-induced MUC5AC overproduction is regulated is still unclear. The focus of the present study was to identify molecular mechanism(s) and regulatory molecule(s) for PGE₂-induced MUC5AC overproduction.

In the present study, we showed that PGE₂ induced MUC5AC mRNA expression in NHNE cells, whereas endogenously produced or endocrinal PGE₂ did not affect MUC5AC gene expression during exogenous PGE₂ treatment (Fig. 1B). Interestingly, even if the half-life of PGE₂ is very short (10), PGE₂ had an effect in the early stage during the 24-h treatment period. The fact that MUC5AC gene expression increases at 12 h after the beginning of PGE₂ exposure can be attributed to PGE₂ binding to its receptors in the early stage. It seems that the kinetics of PGE₂ in the early stage is sufficient to induce MUC5AC gene expression because activated receptor propagates specific signaling to amplify physiological PGE₂ signaling. The biological actions of PGE₂ are mediated by seven transmembrane EP receptors (23). Using RT-PCR, we found that all EP receptor mRNAs were expressed and PGE₂ treatment did not affect EP receptor gene expression in NHNE cells (Fig. 1C). In the airway, EP2 and EP4 receptors have been known as major receptors for MUC5AC gene expression and secretion (14, 20). Consistent with this result, the EP2/4-selective agonist (misoprostol) had a significant effect on MUC5AC gene expression (Fig. 1D), whereas the EP1/3-selective agonist (sulprostone) had no effect. These results suggest that either the simultaneous induction of the two different Gα/H₅₂ couplings PGE₂ receptors plays a pivotal role for PGE₂ during airway mucosal inflammation or both EP2 and EP4 may cooperatively work as a compensatory system for MUC5AC gene expression.

Among these receptors, however, the EP₄-specific antagonist has much more inhibited PGE₂-induced MUC5AC gene expression than the EP₂ antagonist (Fig. 1E). Even if the pharmacokinetics of the two different drugs has been considered deeply, this suggests that EP₂ may be more closely related to PGE₂ signaling at least in part in NHNE cells. Consistent with the pharmacological data, knockdown of EP4 suppressed PGE₂-induced MUC5AC gene expression (Fig. 1F). We wondered what is different between EP₂ and EP₄ despite them both...
being the same G_\text{o}_{2}-coupled receptors. One reason may be due to a difference in their ligand selectivity of PGE_2. Despite almost the same mRNA expression levels of the receptors, cAMP production of the EC_{50} value of EP_4-mediated cAMP production was about 685.1 ± 14.9 pM (Fig. 2A), whereas that of EP_2-mediated cAMP production was 462.33 ± 23.79 pM following PGE_2 treatment (Fig. 2B). This indicates that the higher cAMP concentration of the EC_{50} value of EP_4-mediated cAMP production is because of high selectivity of PGE_2 to convey signaling efficiently to coupled signaling pathways. Consistent with our results, Fujino et al. (11, 12) reported that EP_4 receptor strengthens an association of the PGE_2 to induce T cell factor and early growth response factor-1 in inflammation. Another possible reason that there is a difference in

Fig. 4. RGS4-dependent attenuation of MUC5AC overproduction in the airway. A: 50 μl of 0.1, 1.0, or 5 mg/kg PGE_2 or saline was instilled in the trachea of C57BL/6 mice. Twenty-four hours later, mouse tracheal tissue was processed for real-time PCR and dot blot analysis with anti-MUC5AC IgG (B). Saline was used as a vehicle. *P < 0.05 compared with saline-treated mice. C: 50 μl of 1.0 mg/kg PGE_2 or saline was instilled inside the trachea of the genotyped mice. Twenty-four hours later, trachea was processed for real-time PCR and dot blot analysis with anti-MUC5AC IgG (D). *P < 0.05 compared with PGE_2-treated wild-type mice. E: either lenti::eGFP or lenti::RGS4-eGFP [5 × 10^3 transduction units (TU)/50 μl] was administered drop by drop to the right nostril. Three days after infection, 50 μl of 1.0 mg/kg PGE_2 or saline was instilled inside the trachea. Twenty-four hours later, trachea were processed for PCR. *P < 0.05 compared with PGE_2-treated Rgs4 knock out mice infected by lenti::eGFP. F: genotyped mice were injected with DMSO as a vehicle for different dosages of the EP_4 antagonist, GW627368X (1–5 mg/kg ip), for 1 h before instillation of the EP_4-specific agonist, PGE_1-OH (50 μl of 5 mg/kg it), and PGE_2 (50 μl of 1.0 mg/kg). Trachea from killed mice were harvested for real-time PCR. *P < 0.05 compared with PGE_1-OH-treated wild-type mice. All data shown are representative of 3 independent experiments.
biological actions of EP2 and EP4 may be due to posttranslational modifications, such as glycosylation, hydroxylation, methylation, nucleotidylation, and phosphorylation. Our thinking was supported by several studies that a charged residue(s) in the seventh transmembrane domain and posttranslational modification sites were involved in receptor activation and desensitization (1, 2, 13, 24).

The main finding of this study is that RGS4 suppressed PGE2-induced signaling in vitro and in vivo by enhancing GTPase acceleration. Until now, there has been no report of a relationship between PGE2 receptors and RGS in mucin-related literature. Despite that the characterized main function of RGS proteins is GTPase-activating proteins for Gq/11, Gz11, or G12/13 classes of G proteins, no interaction between RGS and Gz was found (40). Recently, Castellone et al. (4) reported that the Gz-GTP bound axin protein contained an RGS domain, and thus RGS2 was able to promote colon cancer cell growth. In addition, Zheng et al. (40) suggested that the RGS domain of RGS-PX1 specifically interacted with Gz, thereby accelerating its GTP hydrolysis, which leads to an attenuation of Gz-mediated signaling in bovine brain lysates. In the present study, RGS4 bound Gz-GTP, thereby attenuating PGE2-induced MUC5AC overproduction. In addition, the dynamics of heterotrimeric G protein signaling complex are regulated by RGS4 protein in GTP-dependent manner (Fig. 3C). To address the role of RGS4 protein in the pathogenesis of anti-inflammation in the airway, a gain/loss-of-function study in an animal model must be considered. PGE2 instillation into Rgs4 KO mice much increased Muc5ac overproduction compared with that in wild-type mice (Fig. 4, C and D), and Rgs4 function has been restored and Muc5ac gene expression was suppressed after administration of RGS4-expressing lentivirus into Rgs4 KO mice (Fig. 4E). These results demonstrate that RGS4, a classical R4 RGS superfamily lacking any additional well-defined domains (3), negatively regulates the Gz-mediated signaling that induced Muc5ac production during airway mucosal inflammation. These results, however, raise an interesting question. Why does only RGS4 function in the airway epithelium? We just thought that specificity may be mediated in part through selectivity of RGS proteins for particular G protein substrates (17) or that posttranslational modification sites were involved in receptor activation and desensitization (1, 2, 13, 24).

Taken together, these findings suggest that PGE2 may interact directly with EP2 receptor, resulting in increased MUC5AC overproduction. The ability of RGS4 proteins to interact with Gz is important to negatively regulate PGE2-induced MUC5AC overproduction in the airway. As more mechanistic insights are obtained, identification of an RGS accessory molecule that regulates RGS function may be considered.

GRANTS

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