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Loss of Regulator of G protein signaling 5 promotes airway hyperresponsiveness in the absence of allergic inflammation

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Abstract

BACKGROUND—Although eosinophilic inflammation typifies allergic asthma, it is not a prerequisite for AHR, suggesting that underlying abnormalities in structural cells such as airway smooth muscle (ASM) contribute to the asthmatic diathesis. Dysregulation of procontractile, G protein-coupled receptor (GPCR) signaling in ASM could mediate enhanced contractility.

OBJECTIVE—We explored the role of a regulator of procontractile GPCR signaling, RGS5, in unprovoked and allergen-induced AHR.

METHODS—We evaluated GPCR-evoked Ca²⁺ signaling, precision cut lung slice (PCLS) contraction, and lung inflammation in naïve and *Aspergillus fumigatus*-challenged WT and *Rgs5*^{−/−} mice. We analyzed lung resistance and dynamic compliance in live, anesthetized mice by invasive plethysmography.

RESULTS—Loss of RGS5 promoted constitutive AHR due to enhanced GPCR-induced Ca²⁺ mobilization in ASM. PCLS from naïve *Rgs5*^{−/−} mice contracted maximally at baseline, independent of allergen challenge. RGS5 deficiency had little effect on parameters of allergic inflammation including cell counts in bronchoalveolar lavage fluid (BALF), mucin production, ASM mass, and subepithelial collagen deposition. Unexpectedly, induced IL-13 and IL-33 were much lower in challenged lungs from *Rgs5*^{−/−} mice relative to WT.

CONCLUSION—Loss of RGS5 confers spontaneous AHR in mice in the absence of allergic inflammation. Because it is selectively expressed in ASM within the lung and does not promote inflammation, RGS5 may be a therapeutic target for asthma.

Keywords

Asthma; airway hyperresponsiveness; G proteins; Regulator of G protein signaling proteins; *Aspergillus fumigatus*

Introduction

Asthma is characterized by hyperresponsiveness of the airways (AHR) and infiltration of inflammatory leukocytes in the lung, typically associated with allergen exposure ¹. Airway smooth muscle (ASM) contraction is triggered primarily by spasmogens activating G α_q -linked G protein-coupled receptors (GPCRs), including the M3 muscarinic, cysteinyl leukotriene, thrombin, histamine H1, and bradykinin receptors ², and ligands for many of these GPCRs are increased in lungs of allergic asthmatics. Exaggerated bronchoconstriction to the M3R agonist methacholine characterizes asthma ³. GPCR activation induces GTP binding to G α_q , which activates phospholipase C β (PLC β), evoking inositol trisphosphate (IP3) formation, release of Ca²⁺ into the cytosol, cross bridging of the actomyosin complex, and ASM shortening.

Recent work suggests that ASM-intrinsic mechanisms also contribute to AHR independently of inflammation. Mice deficient in the integrin $\alpha 9\beta 1$ had baseline AHR, and tracheal smooth muscle from these mice exhibited increased force generation ex vivo ⁴. Mice lacking a receptor tyrosine phosphatase, CD148, had reduced baseline pulmonary resistance and were protected from allergen-induced AHR ⁵. Underlying dysregulation of ASM procontractile signaling pathways could partially explain the poor efficacy of specific antagonists of individual contraction-inducing receptors in asthma ⁶.

GPCR function is controlled in part by the regulators of G protein signaling (RGS) family that accelerate the intrinsic GTPase activity of G α subunits to attenuate the downstream signaling pathway ⁷. RGS2 was downregulated in peripheral blood mononuclear cells and lungs of asthmatics and in lungs of allergen-challenged mice ⁸, and *Rgs2*^{-/-} mice had spontaneous AHR. However, since RGS2 is widely expressed in many lung constituent cells including epithelium and ASM, the attractiveness of an RGS2-specific therapeutic target for asthma is uncertain.

We found previously that expression of a closely related isoform, RGS5, is restricted to a subset of smooth muscle cells in both humans and mice ⁹. Exposure of cultured human ASM to β -adrenergic agonists, a standard bronchodilator therapy utilized for asthma, reduced RGS5 expression and intensified excitation-contraction responses to GPCR agonists ¹⁰. In a recent study, a single nucleotide polymorphism (SNP) in *RGS5* correlated with clinical response to β -agonists in asthmatic children ¹¹. Here we investigated the effects of RGS5 deficiency on both inflammation and AHR in vivo using *Rgs5*^{-/-} mice. These mice had both spontaneous and inflammation-associated AHR, independent of the degree of inflammation or changes in ASM mass. AHR was principally due to increased ASM excitation-contraction responses to GPCR ligands. These results warrant further investigation into the suitability of RGS5 as a drug target for AHR.

Methods

For complete description of methods, see the Methods section in this article's Online Repository at www.jacionline.org.

Results

RGS5 inhibits GPCR-induced excitation-contraction signaling in mouse ASM

RGS5 overexpression reduced carbachol-elicited bronchoconstriction of human precision-cut lung slices (PCLS) ex vivo⁹, while PCLS from *Rgs5*^{-/-} C57Bl/6 mice bronchoconstricted more to carbachol¹⁰. To determine if augmented excitation-contraction signaling in ASM from RGS5-deficient mice contributed to their increased responsiveness, we examined GPCR-evoked signaling in mouse tracheal ASM (mtASM) cultures from WT and *Rgs5*^{-/-} mice. These cells had similar morphology, growth, and smooth muscle α -actin content (see Fig. E1A in the Online Repository and data not shown). Expression of several pro-contractile GPCRs (Fig. E1B) and downstream signaling components, including phospholipase C β (PLC β), G α_q , G $\alpha_{i1/2}$, G α_{i3} , myosin light chain (MLC), smooth muscle α -actin, and β -arrestin1/2 (Fig. E1C) was similar in WT and RGS5-deficient mtASM. Comparison of *Rgs* expression in mtASM from WT and *Rgs5*^{-/-} mice revealed that *Rgs1* and *Rgs3* were not present, and there was little difference in *Rgs2* expression (Fig. E2A–B). Although *Rgs4* mRNA expression was increased 3–4 fold in mtASM and whole lungs of naïve *Rgs5*^{-/-} mice (Fig. E2A–B), it was decreased in lungs of allergen-challenged RGS5-deficient mice compared to those of challenged WT mice (Fig. E2C). Published studies have noted marked dissociation between RGS4 mRNA and protein levels owing to post-transcriptional regulation^{12, 13}. Accordingly, RGS4 protein amounts were nearly identical in mtASM cells from WT and *Rgs5*^{-/-} mice (Fig. E2D). These results indicate that transcriptional upregulation of *Rgs4* in mtASM and lungs of naïve mice is unlikely to have an impact on AHR in allergen-challenged *Rgs5*^{-/-} mice.

To evaluate excitation-contraction signaling pathways in RGS5-deficient ASM, we treated mtASM cells with various pro-contractile agonists and measured cytosolic Ca²⁺ concentrations by fluorimetry. ACh (Fig. 1A) and bradykinin (BK) (Fig. 1B) elicited significantly more Ca²⁺ flux in mtASM from *Rgs5* knockout mice than WT, particularly at the highest agonist concentrations. In contrast, exposure of WT or RGS5-deficient mtASM to serotonin (Fig. 1C), thrombin (Fig. 1D), thapsigargin, or ionomycin (Fig. 1E) induced comparable Ca²⁺ responses. These experiments suggested that RGS5 inhibits Ca²⁺ signaling induced by some but not all pro-contractile GPCRs in mtASM and that such differences cannot be attributed to alterations in cellular Ca²⁺ stores or Ca²⁺ channel activity.

We next examined events downstream of Ca²⁺ that promote contraction. Because we could not detect robust agonist-induced MLC phosphorylation in these cells, we measured Erk activation as a surrogate for engagement of the contractile apparatus as Erk1/2 regulates MLCK activity and induces Ca²⁺-independent MLC phosphorylation^{14, 15}. Surprisingly, we observed similar basal, ACh, or BK-induced Erk phosphorylation in mtASM cells pooled from WT or *Rgs5*^{-/-} mice by immunoblotting (Fig. E3). Because RGS5 is expressed in a subpopulation of ASM cells in both mouse and human lung⁹, we hypothesized that variable

RGS5 expression in WT cells precluded accurate determination of its role in this signaling pathway using this method. Instead, we evaluated RGS5 expression and Erk1/2 phosphorylation in individual cells using quantitative immunofluorescence¹⁶. RGS5 was expressed in the cytoplasm of some but not all ASM cells (Figs. 2A, E4), and amounts varied 3-fold (Fig. 2C). ACh-induced Erk1/2 activation (pErk staining) was significantly more intense in cells from *Rgs5*^{-/-} mice than in WT cells expressing RGS5 (Figs. 2A–B). ACh-evoked pErk and RGS5 amounts correlated inversely (Fig. 2C). Thus, RGS5 negatively regulates pro-contractile signaling pathways in ASM.

RGS5 regulates lung slice contraction

Lung slices from C57Bl/6 *Rgs5*^{-/-} mice exhibited constitutively increased contraction in response to muscarinic receptor agonists¹⁰. To explore these findings in allergen-induced inflammation and to exclude strain-dependent effects, we backcrossed *Rgs5*^{-/-} mice onto a Balb/c background for 10 generations and compared and evaluated PCLS contraction. Although the baseline diameter of the airways was similar in slices from either strain (Fig. 3A), airways from *Rgs5*^{-/-} mice bronchoconstricted more robustly to CCh (Fig. 3A–B) or ACh (Fig. 3C) than those from WT. RGS5 deficiency primarily affected the efficacy of these muscarinic receptor ligands: the EC₅₀ for carbachol was similar for each strain whereas the E_{max} differed significantly (WT = 37.66 ± 8.665; *Rgs5*^{-/-} = 63.84 ± 7.25, *P* = 0.0001). To determine if the enhanced contraction of PCLS from *Rgs5*^{-/-} mice resulted from intrinsically abnormal signaling responses to these agonists, we measured Erk1/2 phosphorylation. Although baseline pErk levels were similar, ACh-induced pErk was higher in PCLS from *Rgs5*^{-/-} mice than in WT (Fig. 3D).

To investigate mechanisms of RGS5-mediated bronchial contraction in allergic inflammation, we sensitized and challenged mice with extracts of *Aspergillus fumigatus* (*Af*) and measured PCLS contraction. As expected, airways of allergen-challenged WT mice contracted significantly more to carbachol than those from naïve mice (E_{max} in PCLS from challenged mice = 75.24 ± 4.73). In contrast, constriction of PCLS from challenged *Rgs5*^{-/-} mice was not only similar to that of naïve *Rgs5*^{-/-} mice, but also was nearly equivalent to the responses of PCLS from challenged WT mice. These results suggest that airways from mice lacking RGS5 respond maximally to muscarinic receptor stimulation at baseline. In other words, these mice have constitutive AHR that is dissociated from allergen-induced inflammation.

RGS5 modulates the lung cytokine environment of allergen-challenged mice

An alternate mechanism for the equivalent contraction of PCLS from allergen-challenged WT and RGS5-deficient mice could be reduced inflammation in *Rgs5*^{-/-} mice. However, parameters of lung inflammation following sensitization and challenge with *Af*, including total leukocyte numbers (Fig. E5A) and composition (Fig. E5B) in BALF, peribronchial and perivascular inflammation (Fig. E5C), ASM mass (Fig. E5D), goblet cell metaplasia (Fig. E6A), and collagen deposition (Fig. E6B) were similar in naïve and *Af*-challenged WT and *Rgs5*^{-/-} mice. Allergen-associated inflammatory cytokines including IL-4 and IL-5 were similar in BALF from WT and *Rgs5*^{-/-} mice in the presence or absence of *Af* challenge (Figs. 4A–B). Surprisingly, however, IL-13 levels were nearly 3-fold lower in BALF from

allergen-challenged *Rgs5*^{-/-} mice compared to WT mice (Fig. 4C). Although *Il13* mRNA expression increased 10.5-fold in WT lungs following *Af*-challenge relative to naïve mice, *Il13* induction was reduced by more than 40% in *Rgs5*^{-/-} mice ($P = 0.0017$) (Fig. 4D).

IL-33 is a major inducer of *Il13* expression in murine models of asthma^{17, 18}. Human and mouse ASM produce IL-33¹⁹, and *Il13* expression is increased in ASM from severe asthmatics²⁰. We detected a 2.6-fold increase in *Il13* expression in whole lungs of WT *Af*-challenged mice compared to PBS-treated mice (Fig. 4E) whereas upregulation of *Il13* was reduced by 33% in similarly treated *Rgs5*^{-/-} mice ($P < 0.0001$). Recent work indicates that *Aspergillus* components induce proinflammatory cytokine expression through Toll-like receptor (TLR) stimulation^{21, 22}. Accordingly, lipopolysaccharide (LPS, a TLR4 agonist) induced robust *Il13* expression in human or mouse ASM (Figs. 4F, H). TLR-evoked cytokine expression is mediated in part by phosphoinositide-3 kinase (PI3K) signaling²³, and RGS proteins can both positively and negatively regulate PI3K through their interaction with its p85 regulatory subunit²⁴⁻²⁶. Consistent with the hypothesis that TLR-evoked *Il13* expression in ASM is PI3K-dependent, LPS elicited Akt phosphorylation in human or mouse ASM (Fig. 4G and data not shown), and the PI3K inhibitor LY294002 strongly reduced LPS-evoked *Il13* expression (Fig. 4F). LPS induced significantly less *Il13* expression in mtASM from *Rgs5*^{-/-} mice than from WT (Fig. 4F). Surprisingly, overexpression of RGS5 in hASM cells by transduction with tat-RGS5-GFP (Fig. E7) also inhibited LPS-induced *Il13* expression compared to untreated cells or those transduced with tat-GFP alone (Fig. 4H). IL-33 induction in epithelial cells was also decreased in lungs of *Rgs5*^{-/-} mice relative to WT following *Af* challenge (Fig. E8) Taken together, these results indicate that RGS5 levels markedly influence lung IL-33 expression in the context of allergic inflammation.

RGS5 deficiency evokes allergen-independent AHR

To examine how the loss of RGS5 affects pulmonary function in vivo, we measured total lung resistance (R_L) on naïve, anesthetized mice and following methacholine inhalation. Although R_L and dynamic compliance were similar in untreated WT and *Rgs5*^{-/-} mice prior to methacholine treatment, *Rgs5* gene deletion resulted in substantially increased resistance (Fig. 5A) and reduced dynamic compliance (Fig. 5B) in response to a range of methacholine concentrations. We also examined the effects of RGS5 deficiency on *Af*-induced AHR and found little difference between responses to *Af* in WT and *Rgs5*^{-/-} mice (data not shown). These results indicate that the loss of RGS5 evoked spontaneous AHR in untreated mice.

To evaluate the impact of reduced lung IL-13 concentrations on AHR in allergen-challenged *Rgs5*^{-/-} mice, we measured R_L following intratracheal IL-13 instillation. IL-13 did not induce significant changes in inflammatory cell composition in BALF (Fig. E9) but did enhance methacholine-evoked lung resistance compared to saline alone, particularly at the highest methacholine concentration: (R_L saline v. IL-13 in WT mice = 5.16 ± 1.3 cmH₂O.s./ml v. 11.62 ± 3.5 ($P < 0.0001$); R_L saline v. IL-13 in *Rgs5*^{-/-} mice = 7.3 ± 1.5 v. 15.29 ± 3.9 ($P < 0.0001$ for either strain). However, there was no significant difference between IL-13-treated WT and *Rgs5*^{-/-} mice in response to methacholine (Fig. 5C). These

results indicate that lung structural cells respond to IL-13 as expected in the absence of RGS5.

Discussion

This study supports a role for RGS5 in the pathophysiology of asthma. Depletion of RGS5 in mice augments GPCR-triggered Ca^{2+} -signaling, promoting spontaneously increased lung parameters of allergen-induced inflammation (e.g. *Il13* and *Il33* expression) were actually decreased in *Rgs5*^{-/-} mice relative to WT, indicating that the loss of RGS5 enhances AHR specifically by increasing ASM contractility.

Although asthma is considered to be an inflammatory disease typified by eosinophilia and $\text{T}_\text{H}2$ immune responses^{27, 28} and although inflammation promotes AHR²⁹, the diagnosis of asthma can be made in the absence of inflammation. A subset of asthmatics with prominent neutrophilic airway inflammation responds poorly to inhaled corticosteroids³⁰, suggesting that intrinsic abnormalities in lung structural cells including ASM underlie disease pathogenesis in some cases. Although increased expression of proteins of the contractile apparatus has been previously reported in ASM from asthmatics³¹⁻³⁴, we did not detect differential expression of $\text{PLC}\beta$, $\text{G}\alpha_q$, $\text{G}\alpha_{i1/2}$, $\text{G}\alpha_{i3}$ and MLC in mtASM cells from WT and *Rgs5*^{-/-} mice, suggesting that AHR resulted from increased activity of excitation-contraction signaling pathways. Our study suggests that dysregulated bronchoconstrictor signaling due to abnormal RGS protein expression can contribute to AHR in asthma independently of the inflammatory response. Similar uncoupling of airway inflammation and AHR due to ozone has also been reported³⁵.

RGS5 did not affect bronchoconstrictor-induced signaling universally; in fact, RGS5 deficiency augmented Ca^{2+} responses to acetylcholine and bradykinin but had little impact on those to thrombin and serotonin in mtASM. PCLS from *Rgs5*^{-/-} mice contracted more than WT following application of muscarinic receptor ligands acetylcholine and carbachol; however, the poor contractile response of mouse PCLS to agonists other than Ach/CCh, including bradykinin⁹, precluded a more thorough assessment of the regulation of procontractile receptors by RGS5 *ex vivo*. Receptor- or cell type-specific features of RGS proteins could account for this phenotype. A published report demonstrated that RGS5 diminished angiotensin II type 1A but not carbachol-mediated MAPK activation in rat aortic smooth muscle cells³⁶. Second, thrombin (PAR1) and some receptors for serotonin may induce Ca^{2+} flux through activation of both $\text{G}\alpha_q$ and $\text{G}\alpha_{12/13}$ ^{37, 38}. Because RGS5 regulates $\text{G}\alpha_q$ but not $\text{G}\alpha_{12/13}$, the overall responses to these agonists may be minimally affected by the loss of RGS5. Third, RGS5 expression is unequally distributed among ASM cells in mouse and human⁹, which was confirmed in this study. Future work may allow more precise comparison of responses to other procontractile agonists in WT and RGS5-deficient cells through single cell analysis.

Surprisingly, we did not detect increased contraction of lung slices from Af-challenged *Rgs5*^{-/-} mice compared to those from WT mice. Although PCLS from *Rgs5*^{-/-} mice may be maximally responsive to carbachol at baseline, our results suggest that RGS5 also regulates production of IL-13, which augments ASM contractility downstream of GPCRs by

upregulating expression of RhoA and RhoA-kinase (ROCK)^{39,40}. The equivalent responses of WT and *Rgs5*^{-/-} mice to inhaled IL-13 also indicate that RGS5 principally controls GPCR-mediated airway constriction. Moreover, reduced IL-13 quantities in lungs of *Af*-challenged *Rgs5*^{-/-} mice may mitigate the AHR induced by the allergen exposure.

Innate lymphoid type 2 (ILC2) cells appear to be the main source of lung IL-13, and epithelium and ASM-derived IL-33 is a prominent inducer of IL-13 expression in ILC2 cells^{17,19}. *Il13* expression in *Af*-challenged lungs from *Rgs5*^{-/-} mice was significantly lower than controls, suggesting that RGS5 regulates *Il13* expression indirectly through IL-33. Because RGS5 overexpression also inhibited LPS-induced *Il13* expression in ASM, however, it may control this response in a complex manner (Fig. 4F–H). Published studies have indicated that PI3K both positively²³ and negatively⁴¹ modulates LPS-induced proinflammatory cytokine expression, and RGS proteins may enhance or inhibit PI3K activation in various contexts^{24–26}. In addition, recent work has demonstrated that TLR-induced cytokine production is inversely related to Gai2 activity^{42,43}, which is profoundly affected by RGS5 levels.

Further, apoptosis and necrosis promote processing, secretion and activation of IL-33 protein⁴⁴. In a study of mouse ovarian cancer cells in vivo, RGS5 levels correlated with increased survival and increased tumor cell necrosis⁴⁵. Here, we found that ASM cells devoid of RGS5 have enhanced basal viability and increased resistance to necrosis-inducing agents (H₂O₂) than WT counterparts (data not shown), suggesting an additional post-transcriptional mechanism of IL-33 regulation by RGS5. The cause of reduced IL-33 expression in lung epithelial cells of *Rgs5*^{-/-} mice relative to WT (Fig. E8) is less clear because these cells do not express RGS5. We speculate that reduced secretion of IL-33-inducing cytokines (e.g. TNFα) by RGS5-deficient ASM could indirectly attenuate epithelial IL-33 expression.

In contrast to RGS5, RGS2 and RGS4 are highly expressed in epithelial cells^{8,26}. In addition to spontaneous AHR, *Rgs2*^{-/-} mice had increased baseline ASM mass and mucin production. In separate studies, RGS2 inhibited MUC5AC secretion in airway epithelial cells directly⁴⁶ whereas RGS5 did not regulate ATP-induced *Muc5ac* expression in NCI-H292 epithelial cells⁴⁷. We found that RGS5 deficiency did not have a substantial impact on allergen-induced airway remodeling including mucous production, ASM mass, and subepithelial fibrosis in mice, all of which are believed to contribute to AHR in chronic asthma in humans^{48,49}. The selective modulation of ASM contractility by RGS5 in the absence of inflammation suggests that it is a drug candidate for asthma. However, future strategies including genetic targeting or overexpression of RGS5 specifically in ASM in mice and studies of RGS5 expression in subpopulations of asthmatics with varying disease characteristics are needed to fully elucidate its role in the pathophysiology of this disease and its feasibility as a therapeutic target.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Abbreviations used

AHR	airway hyperresponsiveness
GPCR	G protein-coupled receptor
MLC	myosin light chain
PIP2	phosphatidylinositol 4,5-bisphosphate (PIP2)
IP3	phosphatidylinositol 1,4,5-trisphosphate
ERK	Extracellular signal regulated kinase
RGS	Regulator of G protein Signaling
mtASM	mouse tracheal airway smooth muscle cells
PCLS	precision cut lung slices
BALF	bronchoalveolar lavage fluid
M3R	muscarinic receptor 3

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Key messages

- RGS5 is selectively expressed in ASM within lung and negatively regulates procontractile GPCR signaling.
- Loss of RGS5 promotes baseline AHR in the absence of allergen exposure.
- Dysregulated GPCR signaling in ASM may underlie asthmatic endotypes that do not feature prominent inflammation.

Figure 1. RGS5 regulates GPCR-mediated intracellular calcium release

(A–E) Cytosolic calcium release in mtASM from WT and *Rgs5*^{-/-} mice following stimulation with acetylcholine (A), bradykinin (B), serotonin (C) thrombin (D), ionomycin (1 μ M) or thapsigargin (1 μ M) (E). assessed by fluorimetry. RFU values were normalized to the maximal response in (A–D); mean $\pm$ S.E.M from 3–6 independent experiments (in mtASM derived from 10 mice per group) assayed in triplicate (* P < 0.04; ** P = 0.004, unpaired t test).

Figure 2. GPCR-induced Erk activation correlates with RGS5 expression in mtASM

(A) Cells were serum starved for 48 h and stimulated with ACh (10 μ M) for 30 min. Cells were immunostained as indicated. Nuclei were stained with DAPI. (original magnification 63X; scale bar: 10 μ m). (B) Mean fluorescence intensities (MFI) of pErk/ β actin and RGS5 staining (*RGS5*⁺ cells only; mean $\pm$ S.E.M of 30–40 cells/group (**** $P < 0.0001$). (C) MFI of pErk/ β actin staining in a range of WT mtASM cells was plotted against the MFI of RGS5 (43 cells).

Figure 3. RGS5 regulates PCLS contraction independently of allergen challenge (A–C) WT and *Rgs5*^{-/-} mice were sensitized and challenged with PBS or Af followed by measurement of PCLS contraction induced by with carbachol (CCh) (A–B) or acetylcholine (ACh) (C). Arrows show contracting airways; V=vessel; graphs show percent contraction [mean ± S.E.M from 4 mice (25–50 airways in total) per group; ***P* = 0.004, *****P* < 0.0001; n.s., not significant]. (D) Mouse PCLS prepared from WT or *Rgs5*^{-/-} mice were serum starved for 48 h and left untreated (0 min) or stimulated with ACh (10 μM) for the indicated times followed by immunoblotting of lysates with the indicated antibodies.

Figure 4. RGS5 regulates IL-13 production following allergen challenge through IL-33 (A–C) BALF cytokine concentrations (mean $\pm$ S.E.M of 3–8 mice per group; *** P < 0.001; n.s., not significant). (D–E) *Il13* (D) or *Il33* (E) expression in lungs from naïve and *Af*-challenged WT and *Rgs5*^{-/-} mice by qPCR. (F) LPS-induced *Il33* expression in mtASM cells. (G) LPS-induced Akt phosphorylation in hASM cells (representative of 3 independent experiments). (H) LPS-evoked *Il33* expression in hASM cells left untreated (NT) or transduced with tat-GFP or tat-RGS5-GFP. Results in D–F, H are represented as the fold increase relative to naïve or untreated controls, set as ‘1’ (mean $\pm$ S.E.M of 3–8 mice per group (D–E); 2–3 experiments using cells pooled from 7 mice of each genotype (F); or 2 experiments assayed in triplicate (H); *** P = 0.0004, **** P < 0.0001, 1-way ANOVA).

Figure 5. *Rgs5*^{-/-} mice have spontaneous AHR

(A–B) Lung resistance (A) and dynamic compliance (B) in response to increasing doses of aerosolized methacholine (MCh) in naïve BALB/c WT and *Rgs5*^{-/-} mice (mean ± S.E.M of 4 mice per group. * $P < 0.05$; *** $P < 0.001$). (C) Lung resistance in naïve mice following intratracheal instillation of recombinant IL-13 (n = 4 mice per genotype).