Published in final edited form as:

Immunol Res. 2009 ; 43(1-3): 62–76. doi:10.1007/s12026-008-8050-0.

Regulation of G-protein-coupled signaling pathways in allergic inflammation

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Abstract

Abstract Allergic diseases such as asthma are elicited by maladaptive activation of immune cells such as mast cells and lymphocytes by otherwise innocuous allergens. The numerous mediators secreted by such cells promote both acute inflammation and, in many instances, chronic tissue remodeling. Most of these compounds exert their effects on end-organ targets such as epithelial and endothelial cells and airway smooth muscle by activating G-protein-coupled receptors (GPCRs), which are by far the most abundant type of cell surface receptor. Since GPCRs are also the most common target of allergy therapeutics, a better understanding of their intracellular signaling mechanisms is vital to improve the efficacy of such drugs or to develop new targets. In this review, we focus on some of the new regulatory elements that control the duration and amplitude of GPCR signal transduction pathways in immune effector cells and end-organ structural cells affected by allergic inflammation.

Keywords

G proteins; RGS proteins; Signal transduction; Allergy; Asthma; Inflammation; Mast cells; Lymphocytes; Bronchial smooth muscle

Introduction

G-protein-coupled receptors (GPCRs) comprise almost 1% of the human genome and govern how we develop, see, think, and breathe [1]. Over 50% of all pharmaceutical agents currently in use target GPCRs for the treatment of numerous diseases including asthma [2]. The primary signal transducer used by GPCRs is the heterotrimeric G protein, consisting of α , β , and γ subunits [3]. In the absence of receptor ligand, these elements co-exist as a complex in which α subunit is bound to GDP. Upon GPCR stimulation, G_α exchanges GTP for GDP and temporarily dissociates from the $\beta\gamma$ dimer. Each of these activated components interacts with distinct effectors, inducing an array of cellular responses ranging from morphological change to gene transcription [4]. The cycle is terminated by the intrinsic GTPase activity of α subunit, which promotes G_α re-association with $\beta\gamma$ to form an inactive heterotrimer. The rapid turnover between the inactive and the active G protein switches is well suited to precise responses such as hormone secretion or cell movement or shape change. Although $\beta\gamma$ initiates a distinct set of cellular processes, α subunit distinguishes effectors activated by a particular GPCR. There are four major subfamilies of α subunits: α_i , α_s , α_q , and $\alpha_{12/13}$, encoded by 20 α genes [5].

In the immune system, GPCRs play a role in innate, adaptive, and pathological responses. For example, upon exposure to antigens (Ags) after infection or immunization, the chemokine receptors CXCR4 and CXCR5 (and their cognate ligands CXCL12 and CXCL13) facilitate positioning of lymphocytes in lymphoid follicles to create germinal centers (GCs). In these structures, B cells, with help from follicular helper T cells (T_{FH}) cells, proliferate and undergo somatic hypermutation and class switch recombination to secrete high-affinity antibodies [6-9]. Conversely, the serum lipid sphingosine-1-phosphate (S1P) acts through a G_{ai} -coupled GPCR to promote lymphocyte egress from lymphoid organs to the bloodstream [10-12]. In allergic diseases like asthma characterized by chronic inflammation, chemokines secreted by lung resident cells such as bronchial epithelial cells and perhaps mast cells recruit leukocytes including neutrophils and eosinophils [13-15] (Fig. 1). The requirement for GPCR signaling in such processes is illustrated by the fact that chemotaxis induced by chemokines can generally be attenuated by pertussis toxin (PTX), which ADP-ribosylates proteins of the G_{ai} family and subverts GPCR coupling. The invading inflammatory cells and activated lung structural cells produce high quantities of procontractile ligands such as bradykinin, endothelin, and leukotrienes, which act through receptors coupled to G_{aq} [16]. Stimulation of this G-protein leads to the accumulation of cytosolic Ca^{2+} from intracellular pools, which in turn facilitates actomyosin interactions and airway smooth muscle (ASM) contraction.

Physiological regulation of G proteins is mediated in part by the family of regulators of G-protein signaling (RGS) proteins, which number greater than 25 in mammalian cells and can be subdivided into subfamilies on the bases of characteristic domains (Fig. 2a) [17,18]. All RGS proteins contain the characteristic 120 amino acid RGS domain, which mediates binding to G_{α} subunits and GTPase accelerating (GAP) activity. RGS GAP activity accelerates the return of G_{α} to its inactive (GDP-bound) form, promoting more rapid termination of G-protein signaling pathways (Fig. 2b). Although some of the molecular determinants of RGS activity have been defined over the past 12 years since their discovery, much is still unknown about the physiological function(s) of RGS proteins in mammals.

A focal point of research in this field addresses the conundrum that while most cells express several RGS proteins, many RGS family members exert similar GAP activity toward G_{α} proteins in vitro [19]. Such biochemical similarity may or may not signify functional redundancy in vivo. A goal of our laboratory is to understand how RGS proteins modulate specific GPCRs mediating allergic inflammatory diseases. For instance, do RGS proteins regulate chemokine-induced leukocyte infiltration into the lung after allergen exposure? Are there specific RGSs in ASM cells that control bronchial contraction in chronic asthma or other conditions associated with inflammation and airway remodeling? What are the factors that regulate RGS expression and activity? Answers to these questions should elucidate mechanisms and eventually lead to new treatment approaches.

RGS protein regulation of immune cell function

An unexpected role for RGS13 in IgE-mediated allergic responses

Mast cells elicit allergic diseases such as asthma and anaphylaxis characterized by high levels of specific IgE directed against Ags that are otherwise harmless (allergens) [20]. In susceptible individuals, Ag crosslinks IgE bound to the high affinity IgE receptor (FcεRI) on mast cells, which induces release of pre-stored proinflammatory metabolites from granules such as histamine and synthesis of leukotrienes and cytokines. Allergic reactions caused by these mediators are characterized by increased vascular permeability, edema, and smooth muscle contraction.

FcεRI crosslinking by IgE-Ag induces phosphorylation of immunoreceptor-based tyrosine activation motifs in the intracellular portion of the β and γ chains by Lyn tyrosine kinase, which

attracts Syk kinase to the γ chain [21]. Tyrosine phosphorylation of Syk recruits and activates phosphatidyl 3-OH kinase (PI3K), phospholipase $C\gamma$ (PLC γ), and mitogen-activated protein kinase. PI3K has an essential function in mast cell homeostasis and allergic responses [22]. Mast cells from mice expressing a catalytically inactive p110 δ subunit have markedly less Ag-induced degranulation [23]. PI3K-mediated activation of PLC γ , which produces inositol-1, 4, 5 trisphosphate (IP3), evokes a rise in intracellular Ca^{++} concentration leading to degranulation. GPCRs may potentiate IgE-mediated responses or induce mast cell activation acting alone. Stimulation of adenosine (A_3), chemokine, or prostaglandin (EP3) receptors accentuates mast cell degranulation induced by Ag [24]. In contrast to Fc ϵ RI, GPCRs activate the gamma isoform of PI3K. PI3K γ -deficient mast cells have impaired adenosine-mediated degranulation and allergic responses [25]. For these reasons, we investigated RGS proteins as regulators of mast cell-driven allergic inflammation induced by GPCRs.

Human and murine mast cells expressed RGS13, and Fc ϵ RI aggregation upregulated RGS13, suggesting that this RGS protein might also function in Ag-evoked mast cell responses (Fig. 3a) [26]. We generated RGS13-deficient mice with *LacZ* knock-in (referred to as *Rgs13*^{-/-} or *Rgs13*^{LacZ/LacZ}). Both β -galactosidase and immunofluorescent staining of cultured bone marrow-derived mast cells (BMMCs) and tissues showed that RGS13 is expressed in mouse mast cells (Fig. 3b). Unexpectedly, we found that *Rgs13*^{-/-} mice had markedly increased IgE-mediated anaphylactic responses due to more mast cell degranulation (Fig. 3c). Reconstitution of *Rgs13*^{-/-} BMMCs with RGS13 or overexpression of RGS13 in wild-type (WT) BMMCs inhibited Ag-induced degranulation, suggesting that the abnormality in *Rgs13*^{-/-} mast cells was due to the absence of RGS13. Expression of a mutant RGS13 devoid of GAP activity reproduced the effect of WT RGS13, indicating that RGS13 inhibited mast cell degranulation independently of heterotrimeric G-protein binding. Indeed, subsequent biochemical analysis revealed a new and unexpected function for RGS13 in mast cells. RGS13 inhibited mast cell degranulation by physically interacting with the p85 regulatory subunit of PI3K and attenuating Ag-evoked PI3K activation in BMMCs. RGS13 binding to p85 impaired association with an Fc ϵ RI signaling scaffold that includes the adapter protein Gab2.

Thus, these studies uncovered a new signaling molecule, RGS13, which may affect the intrinsic reactivity of mast cells to environmental allergens. As such, abnormalities in RGS13 expression or function could contribute to the pathogenesis of diseases associated with increased mast cell responsiveness in the absence of elevated serum IgE, such as mastocytosis or idiopathic anaphylaxis (IA). IA was first described in 1978 in a series of patients with recurrent anaphylactic episodes where no specific trigger could be identified [27]. IA usually manifests as urticaria, angioedema, wheezing, stridor, and, most importantly, may include hypotension, tachycardia, and sudden cardiac death. Although no offending allergens can be determined in patients with IA, mast cell degranulation, and subsequent release of inflammatory mediators is thought to cause the disease [28]. However, it is unclear whether mast cells from patients with IA have more IgE-mediated degranulation. It is possible that mast cells in IA patients may degranulate more to serum or tissue factors that are independent of IgE.

RGS13 regulates GPCR-mediated functions of human mast cells

As mentioned above, IgE-independent stimuli acting on GPCRs may potentiate IgE-mediated responses or induce mast cell activation acting alone. RGS13 could regulate mast cell responsiveness to allergens through its inhibition of IgE-Ag-induced PI3K activity and to serum components acting on GPCRs through its GAP activity. In order to evaluate the latter possibility, we overexpressed RGS13 in the human mast cell lines HMC-1 and LAD2 or extinguished RGS13 expression by RNA interference. HMC-1 cells depleted of RGS13 had more cytosolic Ca^{2+} in response to several GPCR agonists such as adenosine, CXCL12, and C5a, while RGS13 overexpression inhibited these responses. RGS13-deficient HMC-1 cells

had more chemotaxis and cytokine (IL-8) secretion in response to CXCL12 stimulation than WT counterparts. Finally, LAD2 cells with reduced RGS13 expression degranulated more to S1P. These results demonstrated that RGS13 regulates several G-protein-stimulated events in human mast cells. We are currently studying RGS13 expression and IgE- and GPCR-induced degranulation of human mast cells grown in vitro from peripheral blood progenitors in patients with anaphylaxis and allergy compared to those from healthy controls. These experiments may provide insight into whether RGS13 expression or function is abnormal in IA. Eventually, specific in situ studies may be performed to evaluate mast cell reactivity in these diseases based on in vitro data.

RGS13 regulation of mast cell migration

Since chemokine receptors are coupled to $G_{\alpha i}$, a cognate G-protein substrate of RGS13, this RGS protein may also control mast cell trafficking and chemotaxis. We are currently testing this hypothesis by evaluating the migration of WT and *Rgs13*^{-/-} mast cells in vitro and in vivo. Murine and human mast cells express CCR2 and CCR3, which bind the chemokines MCP-1-5, eotaxin, and RANTES [29,30]. In murine models of airway inflammation, MCP-1 (CCL1) directly activates mast cells, and increased MCP-1 levels have been found in bronchoalveolar lavage fluid (BALF) and bronchial tissue in patients with asthma [31]. Moreover, mast cells from *CCR3*^{-/-} mice mis-localize within the lung after antigen challenge [32]. In order to evaluate the function of RGS13 in mast cell trafficking, we are analyzing chemotaxis of BMMCs from RGS13-deficient mice in response to chemokine stimulation in vitro. To determine how *Rgs13*^{-/-} mast cells migrate in whole animals, fluorescently labeled BMMCs from WT or *Rgs13*^{LacZ/LacZ} mice will be injected into skin, vein, or peritoneum of mast cell-deficient (*Kit*^{W-sh/W-sh}, *W-sash*) mice using established methods [33] followed by enumeration in tissues by histology and flow cytometry. In addition, we will perform bone marrow reconstitution of irradiated *W-sash* mice to analyze the development and localization of transferred mast cells throughout the body and assess responses in models of allergic inflammation.

RGS13 acts as a transcriptional repressor in B lymphocytes

Although some RGS proteins such as RGS2, 3, and 16 are detectable in many different cell types, RGS13 shows restricted tissue expression. In addition to its expression in mast cells, RGS13 is relatively abundant in B lymphocytes. In primary B cells, RGS13 is concentrated in GC B cells [34,35], which are proliferating cells undergoing somatic hypermutation and class-switch recombination. In B cell malignancies, RGS13 is highly expressed in Burkitt lymphomas, which are thought to represent a GC phenotype [34]. In contrast, RGS13 is absent in mantle cell lymphomas, which represent extrafollicular B cells [36]. This expression pattern suggests a potential function of RGS13 in T-cell-dependent antibody responses that occur in the GCs of lymphoid organs after antigen exposure.

Neurotransmitters such as norepinephrine (NE) may regulate such functions of B cells. NE stimulates β_2 -adrenergic receptors on B cells to activate the $G_{\alpha s}$ -cyclic AMP (cAMP)-protein kinase A (PKA) pathway, which induces gene expression through the transcription factor cAMP response element (CRE) binding protein (CREB) [37]. β_2 AR stimulation induces production of cAMP, which activates PKA. PKA phosphorylates CREB, which activates transcription of genes involved in proliferation, hormone secretion, and immunoglobulin (Ig) production, among others, by recruiting co-activators such as CREB binding protein (CBP) or its paralogue p300. In B lymphocytes, CREB stimulates expression of the transcriptional co-activator OCA-B, which is involved in isotype switching to IgE and IgG1 [38,39]. OCA-B is essential for proper immune function as mice deficient in OCA-B do not form adequate GCs in lymphoid organs after immunization [40]. β_2 -receptor stimulation of B cells may also regulate the amount of IgG1 and IgE produced per B cell [41]. Importantly, endogenous

inhibitors of this transcriptional pathway at the level of CREB-coactivator or CREB-DNA interactions have not been previously identified.

Unexpectedly, we found that RGS13 inhibited β_2 AR signaling although RGS13 possesses no GAP activity toward $G_{\alpha s}$ [42]. Overexpression of RGS13 inhibited gene expression evoked by cAMP while knockdown of endogenous RGS13 by siRNA increased transcription of CREB target genes. Further studies revealed that PKA induced translocation of cytosolic RGS13 to the nucleus, where it was captured by phosphorylated CREB (pCREB). RGS13 repressed CREB-dependent gene transcription by directly binding pCREB and reducing its apparent affinity for both the CRE and for CBP/p300. In *Rgs13*^{-/-} B lymphocytes, cAMP-induced pCREB DNA binding and OCA-B expression were increased. Thus, RGS13 inhibits CREB-dependent transcription of target genes through disruption of complexes formed at the promoter. Current work in our laboratory involves examination of the Ig response of B cells to sympathetic stimuli in the presence or absence of RGS13. These studies may further illuminate how the autonomic nervous system influences B-cell-mediated immune functions.

Role of RGS16 in T lymphocyte migration and activation

Chemokines orchestrate coordinated movement of T lymphocytes through lymphoid organs and promote their migration to sites of inflammation [43]. Distinct patterns of chemokine receptors are expressed in naive and activated T cell populations, and gene-targeting experiments have implicated a role for specific chemokines and their receptors in T cell activation, differentiation into T_H1, T_H2, T_H17, T_{REG}, or T_{FH} phenotypes, and inflammatory responses mediated by these subpopulations [44-46]. Asthma is a T_H2-mediated disease in which CD4⁺ and CD8⁺ lymphocytes migrate to the lung parenchyma upon allergen exposure and secrete proinflammatory cytokines (IL-4, -5, and -13), which leads to recruitment and activation of eosinophils [47]. In murine asthma models, T_H2 migration to the lung may be dependent on, among others, CCR3-6, CCR8, and CXCR4 receptors [15,48,49].

The signaling pathways that mediate chemokine-induced trafficking are not completely understood. Although chemokine receptors couple to both $G_{\alpha i}$ and $G_{\alpha q}$, $G_{\alpha i}$ activation appears to be required for chemotaxis in T cells [50,51]. Thymic emigration, transendothelial migration of lymphocytes into lymph nodes and transit through lymphoid organs, and Ag-induced recruitment of lymphocytes to the lung are blocked by PTX, which inactivates $G_{\alpha i}/G_{\alpha o}$ proteins [52]. Consistent with a role for $G_{\alpha i}$ signaling in T cell activation, $G_{\alpha i2}$ -deficient T lymphocytes produce higher levels of proinflammatory cytokines in the gut, which is associated with inflammatory colitis [53]. Whether this abnormality is related directly to $G_{\alpha i}$ function in T lymphocytes is unknown.

Because they directly bind and inactivate $G_{\alpha i}$ and $G_{\alpha q}$ subunits, RGS proteins are poised to regulate chemokine-mediated responses in T cells. Longstanding studies from our laboratory have demonstrated that: (1) T lymphocytes express several RGS proteins, including RGS2 and RGS16. *RGS16* mRNA is increased in human T cells after IL-2 stimulation, whereas RGS2 mRNA is down-regulated by IL-2 [54], (2) RGS overexpression inhibits chemotactic and haptotactic responses in lymphocyte cell lines in vitro [55], and (3) Murine RGS16 is expressed in both T_H1 and T_H2 T cells, and transgenic (Tg) mice expressing RGS16 in CD4⁺ and CD8⁺ lymphocytes had abnormal T cell migration patterns in a model of allergic airway inflammation [56]. T_H2 CD4⁺CCR3⁺ lymphocyte numbers were increased in the spleen of transgenic mice but reduced in BALF. In contrast, RGS16 overexpressing T cells proliferated more and produced more cytokines to Ag stimulation in recall assays, which correlated with increased serum eotaxin levels and airway hyperreactivity (AHR). The molecular mechanisms underlying these observations remain to be determined. In addition, Penninger and colleagues [57] showed that targeted deletion of *Rgs2* in mice resulted in impaired T lymphocyte proliferation and IL-2 production in response to T-cell receptor or PMA stimulation. *Rgs2*^{-/-}

mice also had delayed and reduced responses to lymphocytic choriomeningitis virus infection, which is a model of T_H1-dependent inflammation. These early studies suggested a function of RGS2 and RGS16 in adaptive T cell immune responses in mice.

We are now examining T cell activation and migration to inflammatory stimuli in *Rgs16*^{-/-} mice using models of allergic inflammation. Preliminary data indicate that these mice have similar numbers of lymphocytes in thymus and secondary lymphoid organs as WT mice, suggesting that RGS16 does not play a central role in lymphocyte development or homeostasis. This observation is consonant with the expression of RGS16 primarily in activated lymphoid cells. We are currently studying the response of the mice to immunization with T-dependent Ags and to induction of allergic pulmonary inflammation. Cellular infiltration in BALF and lung tissue preparations are analyzed in OVA-sensitized and challenged mice, and in vivo AHR is assessed by methacholine responsiveness. In addition, contractility of the inflamed airways is determined by direct agonist stimulation of bronchial tissue slices. These studies will provide a greater understanding of how RGS16 affects migration and activation of T lymphocytes and the importance of these cells to the development of allergic airway inflammation.

As mentioned previously, formation of GCs relies on precise chemokine-directed migration of B and T cells, which promotes the temporally and spatially controlled interactions required for maturation of the antibody response to pathogens [6]. BXD2 mice develop autoimmune disease characterized by high levels of serum autoantibodies and immune complex-mediated glomerulonephritis. These mice also have high circulating serum IL-17 levels and develop spontaneous GCs in spleen and lymph nodes, which contain almost entirely CD4⁺IL-17⁺ T cells [58]. Mountz and colleagues found that T_H17-mediated inflammation in BXD2 mice was accompanied by increased expression of both RGS13 and RGS16 in GC B cells. Suppression of IL-17 with Ad-IL-17R:Fc resulted in extinction of RGS13 and RGS16 expression. Importantly, this treatment also eliminated autoantibody production in BXD2 mice, suggesting a role for IL-17 and T_H17 cells in the regulation of GC lymphocyte migration in BXD2 mice through their induction of RGS13 and RGS16 expression. Thus, T_H17 cells and IL-17 together have an important function in the development of autoreactive GCs, and RGS13 and/or RGS16 could regulate localization of lymphocytes providing the impetus for autoantibody production in this model. *Rgs13*^{-/-}/BXD2 or *Rgs16*^{-/-}/BXD2 mice, which are currently being generated, may provide insights into novel pathogenic mechanisms involving IL-17 in the development of autoantibodies observed in autoimmune diseases such as systemic lupus erythematosus (SLE).

RGS16 is expressed in human GC T lymphocytes and inhibits chemokine-mediated migration

We have also examined how RGS16 modulates migration of human T lymphocytes. Burton and colleagues [59] found that RGS16 is enriched in human GC T lymphocytes (CD4⁺CD57⁺). Follicular dendritic cells (FDCs) up-regulate the chemokine receptor CXCR4 on GC T cells, which are adjacent to FDCs in lymphoid tissue. Surprisingly, however, CXCR4^{hi} GC T cells isolated from tonsil responded poorly to CXCL12 ex vivo, whereas CD4⁺CD57⁻CXCR4^{lo} T cells migrated efficiently to CXCL12 in spite of reduced CXCR4 expression. GC T cell non-responsiveness to CXCL12 correlated with high ex vivo expression of RGS13 and RGS16, and FDCs upregulated both RGS13 and RGS16 expression in non-GC T cells. Finally, GC T cells cultured in the absence of FDCs down-regulated RGS13 and RGS16 expression and regained migratory competence to CXCL12. Thus, although GC T cells express high levels of CXCR4, signaling through this receptor appears to be specifically inhibited by FDC-mediated expression of RGS13 and RGS16. These findings provide an example of how fluctuations in RGS protein quantities may modulate chemokine-dependent cell migration and immune cell-cell interactions.

Regulation of lung structural cells by RGS proteins

Because asthma (reversible airways obstruction) is observed in the absence of lung inflammatory infiltrates, airway remodeling, or frank immunological challenge, intrinsic abnormalities in the contractility of ASM may contribute to airway hyperresponsiveness [16, 60]. Natural ligands of GPCRs are the main inducers of ASM contraction or relaxation, and GPCRs are the predominant targets of anti-asthmatic therapy [16]. Mast cell mediators, such as histamine, cysteinyl leukotrienes (e.g., LTD₄), endothelin-1, and bradykinin, act on procontractile GPCRs coupled to G_{αq} to induce bronchoconstriction by facilitating phosphorylation of myosin light chain kinase, which in turn promotes actomyosin interactions (Fig. 4). A requirement for G_{αq} signaling in the lung has been reported for muscarinic receptor-dependent airway responses in a mouse model of allergic asthma [61]. By contrast, G_{αs}-mediated signaling induced by β₂ARs leads to ASM relaxation. Antagonists of CysLT receptors and β₂AR agonists are currently mainstays of asthma treatment. The physiological functions of RGS proteins in the lung are by and large unknown. We are determining which RGS proteins are expressed in specific cell types in the lung to enumerate their functions in normal lung function and in disease processes such as asthma.

Regulation of ASM contractility by RGS4 and RGS5

We detected RGS2, 3, 4, 5, and 10 in cultured mouse and human bronchial smooth muscle cells. In order to define the function of these RGS proteins in ASM, we are using *Rgs4*^{-/-} and *Rgs5*^{-/-} mice and RNA interference in cultured human ASM cells. In the absence of a negative regulator, many GPCR responses are increased in RGS knockout cells due to loss of GAP activity [62,63]. Our preliminary data suggest that cultured ASM transfected with RGS5 siRNA have enhanced Ca²⁺ mobilization in response to several GPCR ligands such as thrombin, endothelin-1, and bradykinin.

Moreover, human asthma is thought to be caused primarily by hyperresponsiveness of the smaller airways (bronchioles), and mechanisms controlling tracheal and bronchial smooth muscle may differ [64]. In order to elucidate how RGS4 and RGS5 regulate the contractility of ASM in vivo, we are analyzing contraction of bronchi from *Rgs4*^{-/-} and *Rgs5*^{-/-} mice by the precision lung slice technique [65]. Lungs are excised and thin slices prepared, which are then cultured in vitro in the presence of semi-solid agarose. After a short period of culture, the agarose is melted, leaving a bronchoalveolar unit tethered to the culture dish. The slice is then treated with agonist and the reduction in bronchiolar diameter (representing contraction) is measured by microscopy. Finally, to determine how RGS4 and 5 expression affects bronchial responsiveness in whole animals, we are evaluating airway reactivity of WT and knockout mice after allergen sensitization and challenge in models of allergic lung inflammation as described above.

In addition to contraction, ASM cells participate actively in the inflammatory process [66]. Airway remodeling in asthma involves, among others, ASM proliferation and hypertrophy in response to growth factors and other inflammatory mediators which may be present in abnormally high amounts [67]. For example, serum factors such as thrombin and S1P stimulate ASM growth [68]. Several compounds like S1P, bradykinin, and thrombin also stimulate cytokine (IL-6) and chemokine (RANTES) release from ASM [69]. We are examining proliferation and synthetic function (e.g., cytokine secretion) of cultured ASM cells from WT and KO mice as well as human ASM depleted of RGS5 by siRNA to determine which responses are regulated by these RGS proteins.

Summary and conclusions

RGS proteins expressed in immune effector cell such as mast cells and lymphocytes as well as in their end-organ targets (i.e., bronchial smooth muscle) represent an important regulatory component of the intracellular signaling pathways induced by GPCRs in allergic inflammation. The role of RGS GAP activity in controlling the intensity of GPCR signaling is also indicated by the hyperresponsiveness of cells expressing G_{α} subunits containing a point substitution that eliminates RGS binding [70]. Thus, inhibition or mimicry of RGS activity may have a profound effect on the potency and/or specificity of GPCR-targeted drugs. A compound that selectively inhibits RGS4 activity has shown initial promise in vitro [71]. Such agents could be more specific than receptor-targeted drugs or even potentiate current therapies, since RGS activity has been shown to increase GPCR agonist potency up to tenfold [72]. In the broadest sense, an understanding of how RGS expression or activity levels modify the development or course of asthma may reveal the importance of a given GPCR in disease pathogenesis. In addition, such studies may one day allow modulation of G-protein signaling downstream of the receptor, creating drugs with better efficacy or fewer side effects.

Acknowledgments

The author thanks Dr. Zhihui Xie, our generous collaborators, and members of the Laboratory of Allergic Diseases for invaluable input and discussion. This work was supported by the Division of Intramural Research, National Institute of Allergy and Infectious Diseases, National Institutes of Health.

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Fig. 1.

GPCRs play a central role in producing the end-organ inflammation in allergic diseases such as asthma. Allergen-specific IgE bound to tissue mast cells induces degranulation and release of proinflammatory mediators in response to crosslinking of the IgE receptor by allergen. Many of these compounds such as histamine and leukotrienes act on GPCRs (represented by the icon that illustrates their seven transmembrane structure) to stimulate lung structural cells. Chemokines and other substances secreted by such cells in turn promote infiltration of T lymphocytes and leukocytes, particularly eosinophils, into the lung. Collectively, these cells produce an inflammatory milieu resulting in hypercontraction of ASM by procontractile ligands of GPCRs, such as bradykinin, endothelin, and leukotrienes.

Fig. 2.

(a) Classification of mammalian RGS proteins. β -Cat, β -catenin-binding; DIX, dimerization domain; D-AKAP, dual-specificity A-kinase anchoring protein; DEP, disheveled/EGL-10/pleckstrin; DH, Dbl homology; GEF, guanine nucleotide exchange factor; GGL, G_{γ} -like; GoLoco, $G_{\alpha i/o}$ -Loco; GRK, G-protein-coupled receptor kinase; GSK, glycogen synthase kinase β -binding; PDZ, PSD95/Dlg/ZO-1/2; PH, pleckstrin homology; PP2A, protein phosphatase 2A; PTB, phosphotyrosine-binding; PX, phosphatidylinositol-binding; PXA, PX-associated; RBD, Ras-binding domain; SNX, sorting nexin. This figure was originally published in Bansal et al. (2008) *Pharm Therap* 116:473. (b) RGS proteins are GTPase activating proteins. GPCR activation induces a conformational change in G_{α} , leading to the

exchange of GTP for GDP. Activated G_{α} (GTP-bound) stimulates downstream effectors. The cycle is terminated by the intrinsic GTPase activity of G_{α} , which facilitates re-formation of the G_{α} -GDP- $\beta\gamma$ heterotrimer. RGS proteins bind to and stabilize a transition state conformation of G_{α} , which greatly accelerates the GTP hydrolysis reaction

Fig. 3.

RGS13 is expressed in mast cells and regulates IgE-dependent degranulation. (a) BMMCs were sensitized with IgE and left untreated or challenged with antigen for 4 or 24 h before cell lysis and evaluation of RGS13 abundance by immunoblotting. (b) Cytosolic expression of RGS13 in BMMCs. The 4-week-old BMMCs from WT or *Rgs13*^{-/-} mice were plated on glass coverslips by cytopspin and immunostained with polyclonal anti-RGS13 followed by Texas Red-conjugated anti-rabbit IgG and DAPI to identify nuclei. (c) WT (solid line) or *Rgs13*^{-/-} cells (dashed line) were stimulated with indicated DNP-HSA concentrations for 30 min prior to measurement of β -hexosaminidase release. This figure was originally published in Bansal et al. (2008) Nat Immunol 9:73

Fig. 4. Mechanisms of GPCR-induced contraction of ASM in asthma. The asthmatic airway is characterized by epithelial sloughing, increased mucus production, and leukocytic infiltration (top panel). Such changes increase the presentation of procontractile ligands acting on (primarily) $G_{\alpha q}$ -coupled GPCRs to hypertrophied bronchial smooth muscle. $G_{\alpha q}$ activation increases cytosolic Ca^{2+} , which results in myosin light chain (MLC) phosphorylation and contraction (bottom panel). Conversely, receptors coupled to $G_{\alpha s}$ (such as the β_2 -adrenergic receptor) inhibit MLC phosphorylation through PKA activation, leading to smooth muscle relaxation