

Molecular mechanisms in allergy and clinical immunology

(Supported by a grant from Merck & Co, Inc, West Point, Pa)

Series editor: Lanny J. Rosenwasser, MD

Heterotrimeric G protein signaling:
Role in asthma and allergic inflammationEric N. Johnson, PhD, and Kirk M. Druey, MD *Rockville, Md*

Asthma and rhinitis are pathophysiologic conditions associated with a prototypical allergic response to inhaled allergens consisting of both neuromechanical and inflammatory components. Heptahelical receptors that bind guanosine triphosphate-binding proteins (G proteins), referred to as *G protein-coupled receptors* (GPCRs), have been intimately linked with asthma and allergic inflammation for many years. G protein signaling mediates responses throughout the immune, nervous, and muscular systems that might contribute to the pathogenesis of allergic processes and asthma. For example, GPCR agonists or antagonists are used as therapies for asthma either by promoting airway smooth muscle relaxation (β_2 adrenergic receptor agonists) or by inhibiting inflammation in the nasal mucosa and airways (cysteinyl leukotriene receptor antagonists). The focus of this review is to explore how downstream signaling cascades elicited by GPCR activation contribute to the allergic phenotype and the mechanism by which pharmaceuticals alter signaling to generate a therapeutic effect. We also discuss physiologic modulators of G protein signaling, such as regulator of G protein signaling proteins and G protein receptor kinases, inasmuch as they represent potential new therapeutic targets in the treatment of atopy and other inflammatory conditions. (*J Allergy Clin Immunol* 2002;109:592-602.)

Key words: Asthma, allergic rhinitis, G proteins, inflammation, signal transduction

Current therapies of asthma and allergic rhinitis are generally targeted to one or the other of 2 components. In asthma, there is a neuromuscular component in which signals generated from neurons and other sources provoke smooth muscle contraction and relaxation. For example, acetylcholine, released from neurons, binds and activates the M3 muscarinic receptor on the surface of smooth muscle cells to elicit contraction. Epinephrine, also released by neurons, is an agonist of the β_2 -adrenergic receptor and stimulates smooth muscle relaxation. The second component of asthma—and the main component of allergic rhinitis—is inflammation. Secretion of media-

Abbreviations used

cAMP:	Cyclic adenosine monophosphate
DAG:	Diacylglycerol
ER:	Endoplasmic reticulum
G protein:	Guanine nucleotide binding protein
GAP:	GTPase activating protein
GDP:	Guanosine diphosphate
GEF:	Guanine nucleotide exchange factor
GPCR:	G protein-coupled receptor
GRK:	GPCR kinase
GTP:	Guanosine triphosphate
MLCK:	Myosin-regulatory light chain kinase
NK:	Neurokinin
PAF:	Platelet-activating factor
PI3K γ :	Phosphoinositide 3 kinase
PKA:	Protein kinase A
PKC:	Protein kinase C
PLC β :	Phospholipase C β
PTX:	Pertussis toxin
RGS protein:	Regulator of G protein signaling
VIP:	Vasoactive intestinal peptide

tors such as histamine and leukotrienes by inflammatory cells, including mast cells, eosinophils, and neutrophils, results in enhanced contractile and proliferative signals to airway smooth muscle in asthma. In both allergic rhinitis and asthma, these mediators induce vasodilation, submucosal edema, and mucus formation. Antagonists of the cysteinyl leukotriene receptors, such as montelukast, have been used to attenuate chemotaxis of inflammatory cells into the lung and might be used to counteract the effect of leukotrienes in allergic rhinitis.¹ Likewise, anticholinergic and antihistaminic agents have been used to offset the enhanced vasodilation and submucosal edema found in allergic rhinitis. These are primary examples of the ability of guanine nucleotide binding protein (G protein) signaling to modulate both the inflammatory component and the neuromuscular component of asthma as well as the inflammation associated with rhinitis (Fig 1).

MECHANISMS OF G PROTEIN SIGNAL
TRANSDUCTION

G protein-coupled receptor ligands

Although the structure of G protein-coupled receptors (GPCRs) is relatively uniform, a tremendous variety of ligands bind these receptors. Prostaglandin and

From the Laboratory of Allergic Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health.

Received for publication November 12, 2001; revised January 2, 2002; accepted for publication January 3, 2002.

Reprint requests: Kirk M. Druey, MD, National Institutes of Health, 12441 Parklawn Dr. Room 200E, Rockville, MD 20852.

Copyright © 2002 by Mosby, Inc.

0091-6749/2002 \$35.00 + 0 1/10/122636

doi:10.1067/mai.2002.122636

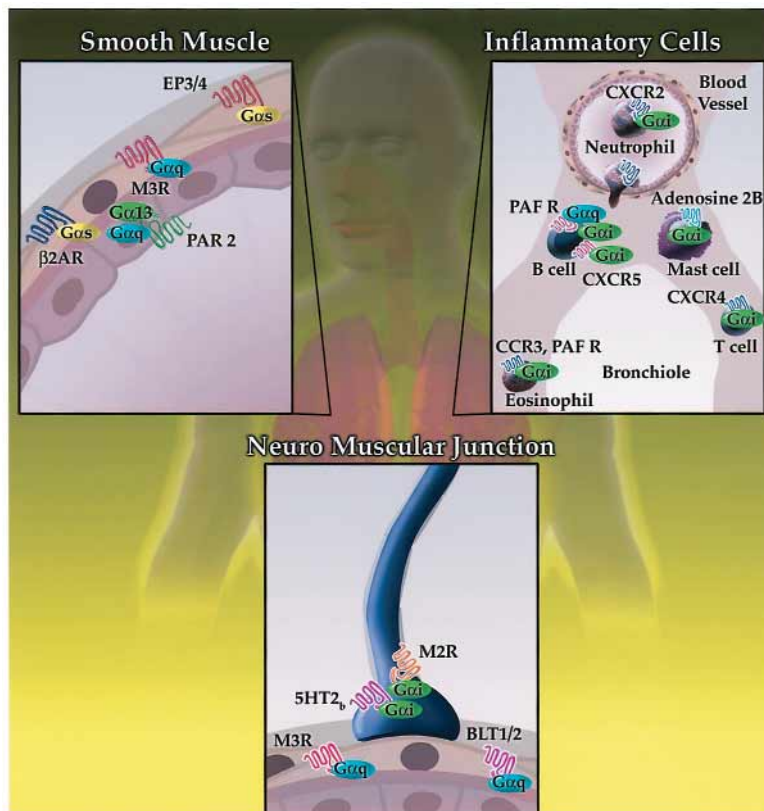


FIG 1. GPCRs and their associated G proteins involved in the generation of allergic responses throughout the respiratory tract. Shown are 3 areas involved in the pathogenesis of asthma: airway smooth muscle, the alveolar submucosa, and the neuromuscular junction. Serpentine receptors that contribute to disease pathogenesis and the $G\alpha$ subunits that couple to them are displayed for these anatomic locations as well as for infiltrating inflammatory cells.

leukotriene receptors are activated by fatty acids. Oligopeptides (fMLP), proteins (chemokines), and neurotransmitters (epinephrine) stimulate GPCRs. The proteolytically activated receptors are activated when a protease, such as thrombin, cleaves a portion of their amino terminus, revealing a tethered peptide ligand, which leads to receptor stimulation. Although there are many different types of agonists that activate GPCRs of similar structure, the specificity of a ligand for its receptor is very high. That is, epinephrine will not activate leukotriene receptors and chemokines will not activate proteolytically activated receptors. Although there are exceptions, this specificity provides one regulatory mechanism for cell signaling.

GPCRs

The mechanism by which GPCRs mediate biological processes has been well characterized over the past 2 decades. The structure of a GPCR is important for its ability to transduce extracellular signals.² GPCRs contain 7 membrane-spanning domains connected by 3 extracellular and 3 cytoplasmic loops. The amino terminus is found outside the cell, whereas the carboxy tail

remains inside the cell. The extracellular loops, as well as extracellular portions of the membrane-spanning α -helices, are often targets for ligand binding to the receptor. The cytoplasmic loops and the carboxy terminus of the receptor are believed to contain both binding sites for the G protein as well as phosphorylation sites that mediate part of a receptor desensitization cascade (see below). GPCR conformation is dynamic. Agonist binding to a GPCR on the outside of the cell stabilizes an “active” receptor conformation and allows the initiation of a signaling cascade inside the cell. Antagonist binding to the GPCR is believed to stabilize an “inactive” conformation and prevent agonist binding. A GPCR in its active conformation catalyzes the exchange of guanosine triphosphate (GTP) for guanosine diphosphate (GDP) on the α -subunit of a heterotrimeric G protein (Fig 2).

The G protein GTPase cycle

Heterotrimeric G proteins consist of α , β , and γ subunits.^{3,4} There are 4 major families of α subunits: $G\alpha_s$, $G\alpha_i$, $G\alpha_q/11$, and $G\alpha_{12/13}$. In the basal state, the α subunit binds GDP and exists as a heterotrimer with $\beta\gamma$. On receptor activation, GDP bound to the α subunit is

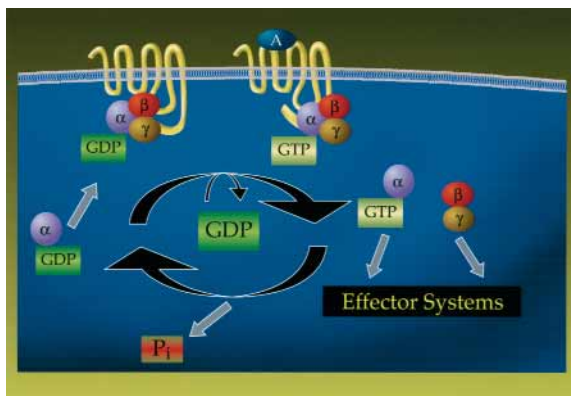


FIG 2. The G protein GTPase cycle. A GPCR is associated with a heterotrimeric G protein consisting of α , β , and γ subunits. In the inactive state, the α subunit binds GDP and is associated with $\beta\gamma$. On agonist (A) binding, the GPCR undergoes a conformational change that stimulates exchange of GTP for GDP on the α subunit. The α subunit then dissociates from $\beta\gamma$, and both α and $\beta\gamma$ activate separate downstream effectors. The cycle is terminated by the enzymatic activity of α , which hydrolyzes GTP to GDP, releasing inorganic phosphate (P_i). GDP-bound α then reassociates with the $\beta\gamma$ subunit.

released and replaced with GTP. GTP-bound $G\alpha$ is believed to separate from the receptor and the $\beta\gamma$ dimer and interact with effector systems,⁵ though there is some evidence that complete dissociation might not occur.⁶ $G\beta\gamma$ interacts with specific effector systems independently of $G\alpha$. Termination of G protein–effector interaction occurs by hydrolysis of GTP to GDP through the intrinsic GTPase activity of the α subunit. GDP-bound $G\alpha$ then reassociates with the $\beta\gamma$ dimer and can be activated on subsequent receptor stimulation. Multiple cycles of GTP binding and hydrolysis occur rapidly (within milliseconds) after receptor stimulation, conferring precision in the response to GPCR agonists.^{6–8}

Cellular responses are determined in part by the identity of the G protein α subunit

The organization of G protein α subunits is classically based on the effectors with which they interact as well as the modifications they elicit (Fig 3). For example, the $G\alpha_s$ family is named for the fact that it stimulates the enzyme adenylyl cyclase, whereas $G\alpha_i$ family members inhibit adenylyl cyclase. Stimulation of phospholipase C β (PLC β) is the result of $G\alpha_q$ activation. Effector systems associated with $G\alpha_{12/13}$ members include the activation of guanine nucleotide exchange factors (GEFs) for the small GTP-binding protein Rho.

G protein effector activation often modifies the concentration of a second messenger. For example, activation of adenylyl cyclase by $G\alpha_s$ results in increased cyclic adenosine monophosphate (cAMP), whereas activation of PLC β by $G\alpha_q$ results in the generation of diacylglycerol (DAG) and inositol tris-phosphate, with subsequent release of Ca^{++} from the endoplasmic reticulum (ER).^{3,9} Stimulation of enzymes can also occur downstream of G protein activation. For example, $G\alpha_q$ -

mediated liberation of DAG stimulates protein kinase C (PKC), whereas $G\alpha_s$ -mediated increases in cAMP activate protein kinase A (PKA). GPCRs thus transduce signals by activating G proteins and modifying second messenger concentration and enzyme activity.

ROLE OF G PROTEIN SIGNALING IN AIRWAY PHYSIOLOGY

Regulation of smooth muscle tone by GPCRs

Contraction of bronchiolar smooth muscle requires an increase in cytosolic calcium. Increases in intracellular Ca^{++} concentration can be mediated by the activation of GPCRs.¹⁰ For example, stimulation of the M3 muscarinic receptor with acetylcholine or BLT1/BLT2 receptors with leukotriene B₄ (LTB₄) activates $G\alpha_q$. $G\alpha_q$ -stimulated PLC β generates IP₃, which binds a receptor on the ER to elicit influx of Ca^{++} from ER stores into the cytosol. This increase in Ca^{++} represents one biochemical mechanism by which stimulation of GPCRs results in smooth muscle contraction. Ca^{++} in smooth muscle cells binds the protein calmodulin to activate the myosin-regulatory light chain kinase (MLCK). Phosphorylation of myosin light chain 20 by MLCK facilitates actin/myosin interaction and activation of myosin ATPase, cross-bridge cycling, and muscle contraction.¹¹ The activation of PKC by DAG is an additional result of $G\alpha_q$ stimulation. Unphosphorylated calponin binds to and prevents activation of the myosin ATPase. PKC-dependent calponin phosphorylation prevents calponin from inhibiting myosin ATPase, suggesting another mechanism by which activation of $G\alpha_q$ might result in smooth muscle contraction (Fig 4, A).¹² Recently published studies have suggested that $G\alpha_{12}$ and $G\alpha_{13}$ might also play a role in smooth muscle contraction. Treatment of rat bronchial smooth muscle with acetylcholine results in activation of RhoA and Ca^{++} sensitization.^{13,14} Rho activation is induced by $G\alpha_{12}$ and $G\alpha_{13}$.¹⁵ In addition, increased expression of $G\alpha_{12}$, $G\alpha_{13}$, and RhoA has been demonstrated in membrane preparations of bronchial smooth muscle from antigen-challenged rats.^{13,14} These data suggest that the relationship between G proteins and asthmatic smooth muscle is not limited to $G\alpha_q$ -mediated smooth muscle contraction and proliferation but might extend to $G\alpha_{12}$ and $G\alpha_{13}$ as well.

Conversely, the use of β_2 adrenergic receptor agonists such as albuterol or stimulation of the prostaglandin E₂ receptor results in the activation of $G\alpha_s$, culminating in smooth muscle relaxation.¹⁶ GTP-bound $G\alpha_s$ stimulates adenylyl cyclase, which catalyzes conversion of ATP to cAMP. Increased intracellular cAMP activates PKA, which in turn phosphorylates several proteins, including MLCK. When MLCK is phosphorylated by PKA, affinity of MLCK for Ca^{++} /calmodulin is decreased, resulting in diminished MLCK activity and ultimately in a decrease in smooth muscle contraction. PKA also phosphorylates Ca^{++} -dependent K⁺ channels, resulting in depolarization and relaxation of smooth muscle, though

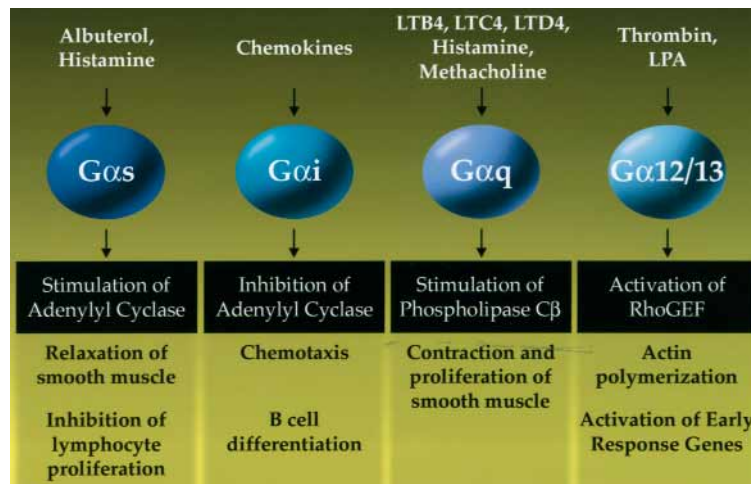


FIG 3. G protein α subunits, their inducers, and their downstream effectors. Heterotrimeric G proteins are classified by their α subunit, divided into 4 subtypes. $G\alpha_s$, which is activated by receptors for such ligands as albuterol and histamine (H₂), stimulates adenylyl cyclase activity, resulting in smooth muscle relaxation and inhibition of lymphocyte proliferation. $G\alpha_i$, which inhibits adenylyl cyclase, is activated by receptors for most chemokines. $G\alpha_q$ activation results from stimulation of leukotriene (CLT1/CLT2, BLT1/BLT2), M3 muscarinic, and histamine (H₁) receptors, causing phospholipase C β activation. The $G\alpha_{12/13}$ family of G proteins is stimulated by receptors for lysophosphatidic acid and thrombin, resulting in actin polymerization and induction of early response gene transcription.

β_2 adrenergic receptor stimulation of Ca⁺⁺-dependent K⁺ channel activity also occurs independently of PKA^{17,18} (Fig 4, B). Phosphodiesterase inhibitors such as theophylline also promote smooth muscle relaxation by directly inhibiting intracellular cAMP metabolism and thereby increasing PKA activity.^{19,20}

In summary, a G protein signaling dichotomy exists in relation to the contractile state of pulmonary smooth muscle in which stimulation of $G\alpha_q$ -mediated pathways evokes contraction while stimulation of $G\alpha_s$ -mediated pathways elicits relaxation.

Potential role of GPCR signaling in airway smooth muscle proliferation

In addition to enhanced bronchial smooth muscle contraction in response to diverse stimuli, a common feature of chronic asthma is hyperplastic/hypertrophic smooth muscle, which might contribute to airway narrowing and increased resistance²¹ and might be mediated by GPCRs. Thrombin, through its receptor, activates phosphoinositide 3 (PI3) kinase, which plays a role in smooth muscle cell proliferation.²² Activation of thrombin or endothelin receptors results in stimulation of airway smooth muscle cell growth, perhaps by activation of a mitogen-activated protein kinase pathway.²³ Elevated levels of endothelin-1 have been reported in bronchoalveolar lavage fluid from asthmatic individuals in comparison with fluid from control individuals.²⁴ GPCRs might also potentiate smooth muscle proliferation induced by epidermal growth factor,²⁵ suggesting that these agents could act in concert to cause the non-neoplastic growth that occurs in diseases such as asthma.

Stimulation of β -adrenergic or prostaglandin E₂ receptors can arrest human cultured airway smooth muscle cells in the G1 phase of the cell cycle.²⁶ Other agents that result in enhanced cAMP levels, such as forskolin, also attenuate airway smooth muscle cell growth. These findings provide alternate mechanisms by which activation of $G\alpha_q$ results in an asthmatic phenotype whereas activation of $G\alpha_s$ counteracts it. Finally, $G\alpha_{13}$ stimulation in smooth muscle cells or resident alveolar macrophages through proinflammatory thrombin or thromboxane receptors might induce expression of nitric oxide synthase, a suspected mediator of airway remodeling through the nitric oxide-mediated generation of oxygen radicals.^{27,28}

Smooth muscle cells might also play a role in the inflammatory aspect of asthma. Smooth muscle cells can act as a source of many chemotactic agents, such as GM-CSF, eotaxin, RANTES, and IL-8.²⁹⁻³² Stimulation of airway smooth muscle cells with IL-1 β and TNF- α results in release of chemokines such as RANTES, eotaxin, IL-8, and GM-CSF,^{29,30} which bind $G\alpha_i$ -coupled GPCRs on eosinophils and neutrophils, recruiting them to the lung. Other studies demonstrate that $G\alpha_s$ -mediated increases in cAMP inhibit the release of eotaxin,²⁹⁻³² RANTES,²⁹ and GM-CSF^{29,30} from smooth muscle cells. Thus, regulation of inflammatory mediator release from smooth muscle cells represents yet another mechanism by which G proteins impact the asthmatic phenotype.

"Neurogenic inflammation," G protein signaling, and asthma

In the classic neuromuscular junction, acetylcholine is released from neurons at the synaptic cleft, where it binds

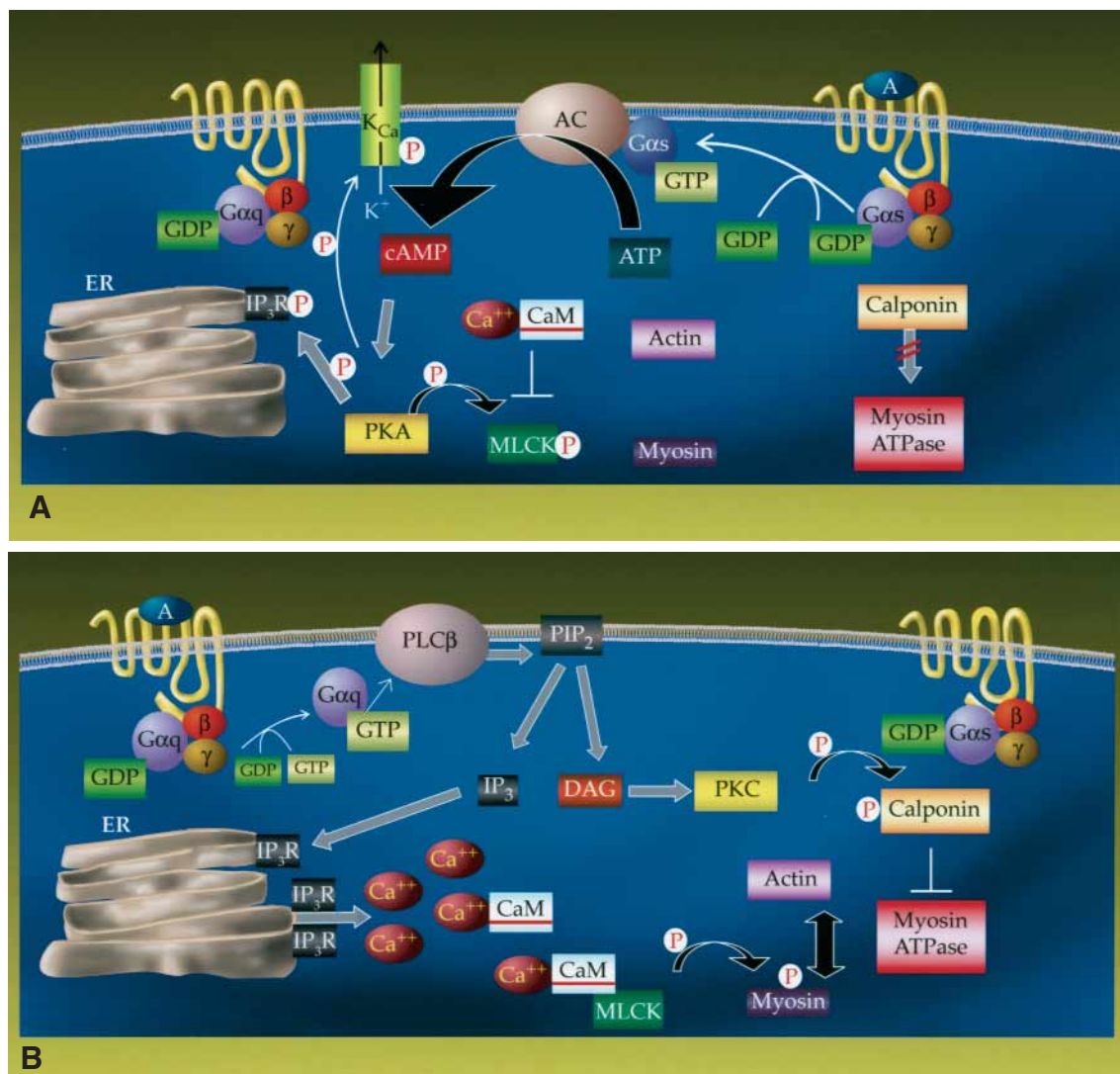


FIG 4. **A**, Signaling events associated with smooth muscle relaxation. Smooth muscle relaxation can be evoked on stimulation of a $G_{\alpha s}$ -coupled receptor—for example, with the β_2 adrenergic receptor agonist albuterol. GTP-bound $G_{\alpha s}$ stimulates adenylyl cyclase, resulting in the conversion of ATP to cAMP. Increased cytosolic cAMP levels activate protein kinase A (PKA), which in turn phosphorylates myosin light chain kinase (decreasing its association with, and therefore activation by, calcium/calmodulin), the IP_3 receptor (decreasing the release of calcium from ER), and the calcium-sensitive potassium channel (resulting in depolarization and relaxation of the smooth muscle). **B**, G protein-mediated signaling associated with smooth muscle contraction. Agonist (methacholine) binding of $G_{\alpha q}$ -coupled receptors such as the M3 muscarinic receptor results in GDP release and GTP binding on the $G_{\alpha q}$ subunit. GTP-bound $G_{\alpha q}$ stimulates phospholipase C β , which cleaves phosphoinositide bisphosphate (PIP_2) into inositol trisphosphate (IP_3) and DAG. IP_3 interacts with IP_3 receptors on the ER and evokes Ca^{++} release from ER stores into the cytosol. Liberated Ca^{++} binds calmodulin, forming a complex (Ca^{++} /calmodulin [CaM]) that activates myosin light chain kinase (MLCK). Phosphorylation of myosin by MLCK facilitates interaction between actin and myosin and smooth muscle contraction. In addition, DAG activates protein kinase C (PKC). Calponin phosphorylation by PKC prevents calponin from inhibiting the myosin ATPase, an alternate mechanism by which $G_{\alpha q}$ stimulation results in smooth muscle contraction.

M3 muscarinic receptors on the surface of smooth muscle cells to elicit contraction through $G_{\alpha q}$ activation. However, acetylcholine binding to $G_{\alpha i}$ coupled M2 receptors on the surface of the postganglionic parasympathetic nerves acts in a feedback inhibitory loop.³³ Thus, by blocking acetylcholine release from neurons, $G_{\alpha i}$ diminishes smooth muscle contraction.

Not only are neuron-derived GPCR ligands able to elicit effects on smooth muscle cells; they can enhance inflammation as well. For example, neurons secrete tachykinins such as substance P, an agonist of the neurokinin (NK) family of GPCRs. Stimulation of NK receptors causes mast cell degranulation, enhanced phagocytosis by macrophages, and eosinophil and neutrophil

activation.³⁴ Asthmatic individuals exhibit increased airway tachykinin and NK receptor levels.^{35,36} An alternate role for GPCRs in neuronal stimulation in asthma is that GPCR agonists, including histamine, serotonin, prostaglandins, and leukotriene B₄, sensitize afferent C fibers, lowering the threshold for neuropeptide release.³⁷

The only neural bronchodilator mechanism in human beings is the inhibitory nonadrenergic noncholinergic (iNANC) nervous system.³⁸ Although members of this class of GPCR ligands, such as vasoactive intestinal peptide (VIP), have been proposed to relax smooth muscle and are effective at lower concentrations than β_2 adrenergic agonists, the role of VIP in asthma is controversial. Whereas some studies have shown the absence of VIP receptors in patients with asthma, others demonstrate that asthmatic individuals exhibit normal receptor expression. Therefore, the role of VIP and the iNANC pathways in asthma remains to be determined.^{16,38}

ROLE OF G PROTEINS IN IMMUNE AND INFLAMMATORY CELLS

Direct effect of G protein signaling on immune cell activation

A hallmark of allergic processes is the infiltration of inflammatory cells into tissues after allergen exposure. B and T lymphocytes, monocytes, neutrophils, mast cells, and eosinophils can be found in the bronchial submucosa and bronchoalveolar lavage fluid of patients with asthma and in nasal secretions from patients with allergic rhinitis. G proteins and their receptors have been demonstrated to play a role in the activation of each of these cell types and in their directed trafficking.³⁹

Many inflammatory cells respond to G protein-mediated signals. T lymphocytes express multiple GPCRs that couple to all 4 G protein classes, including receptors for the serum factors thromboxane A₂, lysophosphatidic acid,⁴⁰ and lysophosphatidylcholine,⁴¹ which can couple to G α_{13} and induce actin polymerization and both basal and directed motility. The T-cell receptor ϵ subunit physically associates with a G protein of the G α_q class (G α_{11}), inducing phospholipase C activation and intracellular Ca⁺⁺ flux.⁴² In addition, another member of the G α_q family, G α_{16} , is expressed exclusively in hematopoietic cells⁴³ and is upregulated by T-cell receptor stimulation.⁴⁴ Expression of dominant negative forms of either G α_{11} or G α_{16} in Jurkat T cells blocks T-cell receptor-stimulated protein tyrosine phosphorylation, Ca⁺⁺ flux, and cytokine secretion.⁴⁵ There is evidence that G proteins regulate B-lymphocyte activation as well. Both G $\beta\gamma$ and G α_q directly stimulate the activity of Bruton tyrosine kinase, a protein that is required for normal B-cell development and activation.^{46,47}

The direct effects of histamine on immune function underscore the importance of G protein signaling for allergic reactions. Histamine, which is secreted at sites of allergic inflammation by mast cells and eosinophils, might influence T-cell differentiation and cytokine secre-

tion. Previous studies suggest that H₁R stimulation augments antigen stimulation of T lymphocytes.⁴⁸ T_H1 CD4⁺ lymphocytes express predominantly H₁ receptors, which are coupled to G α_q , whereas T_H2 cells express H₂ receptors, coupled to G α_s . Studies of mice lacking either H₁ or H₂ receptors suggest a direct antiallergic effect of secreted histamine in allergic reactions. H₁ receptor stimulation upregulates T_H1 responses, whereas both T_H1 function and T_H2 function are suppressed by H₂ receptor activation.⁴⁹ It is unclear whether the anti-inflammatory effect of G α_s on T cells is specific to H₂ receptors, because β_2 AR stimulation of G α_s in T lymphocytes does not influence T_H2 function (IL-4 production).⁵⁰ A potential mechanism to explain this discrepancy is differential expression or specificity of regulators of these 2 pathways, such as the GPCR kinases (GRKs), which are discussed below. Histamine might also modulate inflammatory responses in allergic processes such as atopic rhinitis by affecting chemokine expression. One recent study found that both histamine and allergen upregulate chemokine mRNA expression in explant cultures of human nasal mucosa.⁵¹

In addition to its role in cell recruitment to inflammatory locations, GPCR signaling might also directly impact the function of allergic inflammatory cells. For example, basophils degranulate in response to chemokines, releasing histamine and leukotriene C₄.⁵² Eotaxin, which is the predominant chemoattractant for eosinophils, markedly enhances antigen-induced IL-4 production in basophils.⁵³ ATP induces calcium flux in both eosinophils and neutrophils.⁵⁴ Although this effect is believed to be mediated by the PY2 purinergic receptor expressed in both cell types, differential sensitivity to pertussis toxin (PTX), an inhibitor of G α_i/o proteins,³ suggests that the signaling cascade in each cell might be different. Distinct PY2 receptor subtypes coupled to unique G proteins could explain this difference. Mast cells release adenosine on degranulation and express adenosine GPCRs,⁵⁵ indicating a potential autocrine mechanism for mast cell regulation.

Leukotrienes are potent chemotactic agents and play a direct role in the pathogenesis of asthma and allergic rhinitis. The cysteinyl leukotrienes C₄, D₄, and E₄ bind 2 closely related receptors, CLT1 and CLT2, expressed on leukocytes, smooth muscle cells, and macrophages.⁵⁶ CLT stimulation induces Ca⁺⁺ flux through a PTX-resistant G protein (probably G α_q), resulting in smooth muscle contraction and proliferation as well as eosinophil recruitment and edema in the submucosa of the lung and nose. In contrast, leukotriene B₄ binds a separate pair of receptors, BLT1 and BLT2, on neutrophils and macrophages, which induce chemotaxis, Ca⁺⁺ flux, and IP₃ production through both G α_{16} and G α_i but not G α_q .⁵⁷⁻⁵⁹

Platelet-activating factor (PAF), a phospholipid that has been implicated in allergy pathogenesis, activates a GPCR expressed in B lymphocytes, eosinophils, and platelets and is secreted by eosinophils. PAF induces eosinophil chemotaxis, degranulation, and generation of superoxide anion, which depend on G α_i activation inasmuch as these responses are blocked by PTX.⁶⁰ Further-

more, degranulation or superoxide anion generation provoked by either IgG receptor or IL-5 stimulation of eosinophils is also inhibited by PAF antagonists, implying that cytokine or Fc receptor eosinophil signaling might be regulated in an autocrine/paracrine manner by PAF.

In summary, GPCRs not only influence inflammatory cell function directly through their own signaling pathways but might also modulate signaling through antigen or cytokine receptors, either by causing release of cytokine ligands or potentially by coupling G proteins or effectors to the antigen or cytokine receptor or a downstream component of their signaling pathways. Such a paradigm is established in other cell types, such as fibroblasts and epithelial cells, in which GPCR stimulation results in transactivation of receptor tyrosine kinases such as the epidermal growth factor receptor, which are not G protein-linked.⁶¹

Role of G proteins in T_H1 and T_H2 inflammation: Studies of normal and genetically altered mice

Animal models both in normal and knockout mice lacking one or more components of G protein signaling pathways suggest a role for G protein signal transduction in both T_H1 and T_H2 inflammation. For example, transgenic overexpression of the G α i/o inhibitor PTX in murine lymphocytes preserves T-cell activation responses but results in accumulation of mature thymocytes in the thymus and underpopulation of secondary lymphoid organs,^{62,63} suggesting that a G α i-coupled signal transduction pathway controls thymocyte emigration. In recent years, the discovery and characterization of chemokines has confirmed the importance of G α i-coupled signaling for both B- and T-cell migration to and organization within secondary lymphoid compartments. Further control of inflammatory cell recruitment to sites of inflammation or lymphoid organs is exerted by differential induction of chemokines in response to specific allergens or pathogenic stimuli.^{64,65} For example, the chemokine SDF1 α (CXCL12), which binds the G α i-coupled receptor CXCR4 in T lymphocytes, is the most potent known chemoattractant for these cells. CXCL12 neutralization by specific antibodies has been shown to reduce lung inflammation and airway hyperresponsiveness in a mouse model of allergic airway disease.⁶⁶ A recent study found elevated serum levels of several chemokines, such as RANTES and eotaxin, in the serum of patients with atopic dermatitis.⁶⁷ Another chemokine, TARC (thymus and activation-regulated chemokine) is expressed predominantly by human dermal epithelial cells. In atopic dermatitis, the proportion of T lymphocytes expressing the TARC receptor (CCR4), which are mostly of the T_H2 phenotype, is increased in lesional skin, suggesting that TARC could play a role in the development of T_H2-mediated inflammation in this disease.⁶⁸

Recently published data also suggest that chemokine signaling might influence T-cell differentiation and polarization directly. PTX has long been known to pro-

mote T_H1 responses, such as experimental allergic encephalomyelitis.⁶² Another example of how G α i signaling might influence T-cell polarization is chemokine receptor CCR1-deficient mice, which display a shift toward T_H2 T-cell differentiation in response to pathogens that normally provoke a T_H1 phenotype.⁶⁹ One potential explanation for these abnormalities is altered polarizing cytokine (eg, IL-12) production by antigen-presenting cells as stimulation of both G α i-coupled (chemokine) and G α s-coupled (PGE₂, β -adrenergic) receptors on monocytes inhibits IL-12 production, a cytokine critical for the differentiation of CD4⁺ cells to a T_H1 phenotype.⁷⁰

Consistent with a role for G α i2 in T_H1 cell development and inflammatory responses, G α i2-deficient mice develop severe inflammatory bowel disease,^{71,72} which is associated with increased local production of T_H1 cytokines (IL-12, IFN- γ , TNF- α) in the gut.⁷³ In addition, these mice show an increase in mature thymocytes in the thymus, similar to what is seen in PTX transgenic mice. However, unlike PTX transgenic mice, G α i2 $-/-$ animals display normal numbers of T cells in the spleen and lymph nodes. These results suggest that another PTX-sensitive G protein, such as G α i3, might be involved in T-cell homing to these organs. Thus, differential coupling of chemokine receptors to closely related G proteins could determine appropriate trafficking to lymphoid compartments and sites of inflammation.

Deletion of the G protein effector PI3 kinase γ (PI3K γ), which is thought to link both G α i and G α q receptor stimulation to gene transcription and cellular proliferation, illuminates an important role for this molecule in inflammation. Neutrophils from PI3K γ $-/-$ mice exhibit aberrant responses to a variety of chemoattractants, including diminished IP₃ production, respiratory burst, and chemotaxis.^{74,75} Moreover, PI3K γ -deficient neutrophils and macrophages fail to accumulate at sites of inflammation in a septic peritonitis model. Given that PI3K γ is activated in response to both G α i- and G α q-coupled receptor stimulation,⁷⁶ GPCR signaling could play a primary role in the attraction of a variety of immune cells to sites of inflammation.

Finally, mice deficient in Lsc, an effector of G α 12 and 13, reveal a central role for this G protein signaling pathway in T- and B-cell function.⁴⁰ Lsc contains a Dbp-homology domain, which stimulates GTP binding by the small G protein Rho, and Rho plays a central role in reorganization of the actin cytoskeleton.¹⁵ Lsc also contains a "RhoGEF-RGS (rgRGS)" domain, which confers the ability to act as a GTPase activating protein (GAP) on G α 12 and G α 13 (see below). Through its GAP activity, Lsc might negatively regulate signaling mediated by these G proteins. Lsc-deficient lymphocytes demonstrate dramatically reduced motility in response to lysophosphatidic acid or thromboxane, agonists that stimulate G α 13-coupled receptors, but exhibit normal responses to chemokine stimulation. In addition, Lsc $-/-$ mice demonstrate dysregulated trafficking of T lymphocytes to secondary lymphoid organs and, in particular, an absence of

Image available in print only

FIG 5. A, RGS proteins are GTPase-activating proteins for $G\alpha$ subunits. RGSs bind activated $G\alpha$ and enhance the intrinsic GTPase activity of the α subunit. The lifetime of active $G\alpha$ is thus shortened, decreasing downstream effector stimulation. **B,** GRKs promote “homologous” receptor desensitization by phosphorylating only active (agonist-bound) receptors. The phosphorylated receptor recruits a second protein, β -arrestin, which in turn assists in clathrin-mediated endocytosis of the receptor. (Figure adapted with permission from: Nature Reviews Neuroscience 2001;2:728. Copyright 2001 Macmillan Magazines Ltd.)

marginal zone B cells in the spleen. Thus, $G\alpha_{12}$ and $G\alpha_{13}$ likely play a role in the motility and homing of both T and B lymphocytes.

REGULATION OF POST-RECEPTOR G PROTEIN SIGNALING: A POTENTIAL NEW TARGET OF ANTIALLERGY THERAPY

The pharmaceutical industry has made tremendous progress in the treatment of allergic disease with GPCR agonists and antagonists such as β agonists. However, these therapies are not without drawbacks. For example, long-term usage of β_2 adrenergic receptor agonists might result in a downregulation of both receptor and $G\alpha$ s protein, leading to a decreased efficacy of the drug.⁷⁷ Pathologic inflammatory responses in the absence of normal GPCR signaling (as revealed by studies of knockout mice) suggest that therapeutic agents targeting the G protein signaling cascade downstream of the GPCR warrant further study. In addition, the cell has developed its own physiologic mechanisms to prevent naturally occurring, chronically enhanced signaling, including the regulators of G protein signaling (RGS proteins) and GRKs.

RGS proteins

Endogenous regulation of GPCR cascades has recently been elucidated with the discovery of the RGS protein family. There are more than 20 members of the RGS family in mammals; there are also members in yeast, *Drosophila*, and other organisms.⁷⁸ The putative function of RGS proteins is to attenuate G protein signals. This inhibition is accomplished by 2 distinct mechanisms. First, RGS proteins bind to $G\alpha$ subunits of the $G\alpha_i$, $G\alpha_q$, and $G\alpha_s$ classes and stabilize a conformation that favors GTP hydrolysis (Fig 5).^{8,79-81} Therefore, the duration of interaction of GTP-bound $G\alpha$ with its effectors is shorter in the presence of an RGS protein than it is in an RGS-

free environment. The ability to increase the rate of GTP hydrolysis is termed *GAP activity*. Hastened hydrolysis of $G\alpha$ -GTP results in a more rapid production of $G\alpha$ -GDP, which rebinds $G\beta\gamma$. Therefore, GAP activity shortens not only the activation half-life of $G\alpha$ but that of $G\beta\gamma$ as well. The second mechanism by which RGS proteins attenuate G protein-mediated signal transduction is effector antagonism. That is, some RGS proteins block the ability of G proteins to interact with effector molecules, independently of GAP activity.⁸²

There is preliminary evidence that RGS proteins might regulate the function of immune and inflammatory cells. RGS1 and RGS3 are expressed in monocytes and B cells, whereas RGS2, RGS3, RGS14, and RGS16 are expressed in T lymphocytes.⁸³⁻⁸⁸ Overexpression of RGS1 or RGS3 but not RGS2 impairs chemotaxis in response to several chemokines in the B-cell line 2PK3.⁸⁸ Various GPCR stimuli, such as PAF, upregulate RGS1 protein levels in B lymphocytes; RGS1, in turn, inhibits PAF-induced mitogen-activated protein kinase activation in B lymphocytes when stably overexpressed.⁸³ These data suggest that in some cases RGSs might be induced by G protein signals in a feedback loop to “desensitize” the receptor after sustained agonist stimulation. Furthermore, antigen receptor stimulation of B cells induces expression of RGS1 and RGS3 while downregulating RGS2.⁸⁸ Similarly, IL-2 evokes RGS16 expression while decreasing RGS2 mRNA in T lymphocytes.⁸⁹ Thus, regulation of RGS protein expression might represent another intersection between signaling mediated by cytokines or immune receptors and heterotrimeric G proteins. Although the physiologic role of RGS proteins is uncertain, preliminary observations of RGS2 knockout mice provide evidence of their importance in inflammatory cell function. RGS2 $-/-$ mice demonstrate impaired T-cell proliferation and IL-2 production after T-cell receptor stimulation as well as defective antiviral immunity *in vivo*.⁹⁰ However, the biochemical basis for these abnormalities remains to be determined.

Although it is in its infancy, the characterization of RGS proteins could reveal a potential target for therapeutic intervention in asthma. For instance, if a drug mimicked RGS protein inhibition of $G_{\alpha q}$ in specific pathways, airway smooth muscle cell contraction would theoretically be attenuated. Similarly, if an RGS protein possessing GAP activity toward $G_{\alpha i}$ were introduced into inflammatory cells, migration of these cells to inflammatory sites might be diminished.

GRKS

A second mode of GPCR downregulation exists in the form of GRKs. Seven GRKs have been cloned thus far, including GRK2 and GRK3, which are expressed in lymphocytes. These molecules mediate "homologous" desensitization of GPCRs, inasmuch as only agonist-bound receptors are substrates for GRK phosphorylation.^{91,92} In contrast, "heterologous" desensitization of GPCRs can be mediated by kinases such as PKA, which might be induced by a variety of signals. GRK phosphorylation of the receptor recruits an additional molecule, termed β -arrestin. Arrestin recruits the receptor to clathrin-coated pits, leading to dynamin-dependent internalization of the receptor to endosomes.

GRKs have been implicated in the pathogenesis of inflammation. GRK5 knockout mice demonstrate enhanced responsiveness to muscarinic receptor stimulation.⁹² β -arrestin 2 knockout mice fail to develop tolerance to repeated doses of morphine, the agonist of the μ -opioid GPCR, and because the related protein β -arrestin 1 regulates β 2AR, one can extrapolate a role for β -arrestin 1 in the tolerance to chronic exposure to β -agonists. Another study demonstrated decreased levels of GRK2 and GRK3 in lymphocytes extracted from synovial fluid of patients with rheumatoid arthritis.⁹³ If chemokine receptors expressed in rheumatoid arthritis lymphocytes are supersensitive to stimulation, this G protein signaling abnormality could contribute to enhanced recruitment of inflammatory cells to the synovium.

CONCLUSION

In summary, the importance of GPCR signaling for airway smooth muscle contraction in asthma and for inflammation associated with allergic diseases such as rhinitis and atopic dermatitis has long been recognized. More recently, the characterization of signaling pathways evoked by chemokines has led to the discovery of new roles for GPCRs in the activation and differentiation of immune effector cells and in the recruitment of these cells to sites of inflammation. With few exceptions, pharmacologic treatment of allergic disease targeting G protein signaling has been limited primarily to specific stimulation or inhibition of GPCRs. However, to date, G protein signaling events subsequent to receptor activation in the pathogenesis of allergic/ T_H2 inflammation have not been well studied. The discovery of physiologic regulators of G protein signaling, the RGS proteins and GRKs, suggests that further investigations of these cas-

cases is warranted, inasmuch as they might lead to identification of compounds that modulate responsiveness of G protein signaling pathways beyond the level of the receptor. Such novel therapeutic agents could be more specific and cause fewer side effects.

We thank Drs Eric Lippert, Andrea Keane-Myers, and J. Paul Justice for critical discussions and reading of the manuscript.

REFERENCES

- DuBuske LM. Mediator antagonists in the treatment of allergic disease. *Allergy Asthma Proc* 2001;22:261-75.
- Gether U, Kobilka BK. G protein-coupled receptors II. Mechanism of agonist activation. *J Biol Chem* 1998;273:17979-82.
- Gilman AG. G proteins: transducers of receptor-generated signals. *Ann Rev Biochem* 1987;56:615-49.
- Neer EJ. Heterotrimeric G proteins: organizers of transmembrane signals. *Cell* 1995;80:249-57.
- Hamm HE. The many faces of G protein signaling. *J Biol Chem* 1998;273:669-72.
- Chidiac P, Markan VS, Ross EM. Kinetic control of guanine nucleotide binding to soluble $G_{\alpha q}$. *Biochem Pharmacol* 1999;58:39-48.
- Ross EM. G protein GTPase-activating proteins: regulation of speed, amplitude, and signaling selectivity. *Recent Prog Horm Res* 1995;50:207-21.
- Mukhopadhyay S, Ross EM. Rapid GTP binding and hydrolysis by $G_{\alpha q}$ promoted by receptor and GTPase-activating proteins. *Proc Natl Acad Sci U S A*. 1999;96:9539-44.
- Berridge MJ. Inositol lipids and cell proliferation. *Biochim Biophys Acta* 1987;907:33-45.
- Hakonarson H, Grunstein MM. Regulation of second messengers associated with airway smooth muscle contraction and relaxation. *Am J Respir Crit Care Med* 1998;158:S115-122.
- Sweeney HL. Regulation and tuning of smooth muscle myosin. *Am J Respir Crit Care Med* 1998;158:S95-S99.
- Webb BLJ, Hirst SJ, Gienbycz MA. Protein kinase C isoenzymes: a review of their structure, regulation and role in regulating airway smooth muscle tone and mitogenesis. *Br J Pharmacol* 2000;130:1433-52.
- Chiba Y, Misawa M. Increased expression of G12 and G13 proteins in bronchial smooth muscle of airway hyperresponsive rats. *Inflamm Res* 2001;50:333-6.
- Chiba Y, Takada Y, Miyamoto S, Mitsui-Saito M, Karaki H, Misawa M. Augmented acetylcholine-induced, Rho-mediated Ca^{++} sensitization of bronchial smooth muscle contraction in antigen-induced airway hyperresponsive rats. *Br J Pharmacol* 1999;127:597-600.
- Sah VP, Seasholtz TM, Sagi SA, Brown JH. The role of Rho in G-protein coupled receptor signal transduction. *Ann Rev Pharmacol Toxicol* 2000;40:459-89.
- Thirstrup S. Control of airway smooth muscle tone, II: pharmacology of relaxation. *Respir Med* 2000;94:519-28.
- Kume H, Hall IP, Washabau RJ, Takagi K, Kotlikoff MI. Beta-adrenergic agonists regulate K_{Ca} channels in airway smooth muscle by cAMP-dependent and independent mechanisms. *J Clin Invest* 1994;93:371-9.
- Kume H, Takai A, Tokuno H, Tomita T. Regulation of Ca^{++} -dependent K^{+} -channel activity in tracheal myocytes by phosphorylation. *Nature* 1989;341:152-4.
- Niewoehner DE, Campe H, Duane S, McGowan T, Montgomery MR. Mechanisms of airway smooth muscle response to isoproterenol and theophylline. *J Appl Physiol Respir Environ Exercise Physiol* 1979;47:330-6.
- Newman DJ, Colella DF, Spainhour CB Jr, Brann EG, Zabko-Potapovich B, Wardell JR. cAMP-phosphodiesterase inhibitors and tracheal smooth muscle relaxation. *Biochem Pharm* 1978;27:729-32.
- Chiappara G, Gagliardo R, Siena A, Bonsignore MR, Bousuet J, Bonsignore G, et al. Airway remodeling in the pathogenesis of asthma. *Curr Opin Allergy Clin Immunol* 2001;1:85-93.
- Krymskaya VP, Penn RB, Orsini MJ, Scott PH, Plevin RJ, Walker TR, et al. Phosphatidylinositol 3-kinase mediates mitogen-induced human airway smooth muscle cell proliferation. *Am J Physiol* 1999;277:L65-L78.
- Shapiro PS, Evans JN, Davis RJ, Posada JA. The seven-transmembrane-spanning receptors for endothelin and thrombin cause proliferation of air-

- way smooth muscle cells and activation of the extracellular regulated kinase and c-junNH2-terminal kinase groups of mitogen-activated protein kinases. *J Biol Chem* 1996;271:5750-4.
24. Trakada G, Tsourapis S, Marangos M, Spiropoulos K. Arterial and bronchoalveolar lavage fluid endothelin-1 concentrations in asthma. *Respir Med* 2000;94:992-6.
 25. Krymskaya VP, Orsini MJ, Eszterhas AJ, Brodbeck KC, Benovic JL, Panettieri RA, et al. Mechanisms of proliferation synergy by receptor tyrosine kinase and G protein-coupled receptor activation in human airway smooth muscle. *Am J Respir Cell Mol Biol* 2000;23:546-54.
 26. Stewart AG, Harris T, Fernandes DJ, Schachte LC, Koutsoubos V, Guida E, et al. Beta2-adrenergic receptor agonists and cAMP arrest human cultured airway smooth muscle cells in the G(1) phase of the cell cycle: role of proteasome degradation of cyclin D1. *Mol Pharmacol* 1999;56:1079-86.
 27. Kitamura K, Singer WD, Star RA, Muallem S, Miller RT. Induction of inducible nitric-oxide synthase by the heterotrimeric G protein Gα13. *J Biol Chem* 1996;271:7412-5.
 28. Trifilieff A, Fujitani Y, Mentz F, Dugas B, Fuentes M, Bertrand C. Inducible nitric oxide synthase inhibitors suppress airway inflammation of mice through downregulation of chemokine expression. *J Immunol* 2000;165:1526-33.
 29. Hallsworth MP, Twort CH, Lee TH, Hirst SJ. β2-adrenoceptor agonists inhibit release of eosinophil activating cytokines from human airway smooth muscle cells. *Br J Pharmacol* 2001;132:729-41.
 30. Lazzeri N, Belvisi MG, Patel HJ, Yacoub MH, Fan Chung K, Mitchell JA. Effects of prostaglandin E2 and cAMP elevating drugs on GM-CSF release by cultured human airway smooth muscle cells. Relevance to asthma therapy. *Am J Respir Cell Mol Biol* 2001;24:44-8.
 31. Ammit AJ, Hastie AT, Edsall LC, Hoffman RK, Amrani Y, Krymskaya VP, et al. Sphingosine 1-phosphate modulates human airway smooth muscle cell functions that promote inflammation and airway remodeling in asthma. *FASEB J* 2001;15:1212-4.
 32. Pang L, Knox AJ. Regulation of TNF-α-induced eotaxin release from cultured human airway smooth muscle cells by beta2-agonists and corticosteroids. *FASEB J* 2001;15:261-9.
 33. Fryer AD, Jacoby DB. Muscarinic receptors and control of airway smooth muscle. *Am J Respir Crit Care Med* 1998;158:S154-S160.
 34. Kraneveld AD, James DE, deVries A, Nijkamp FP. Excitatory non-adrenergic-non-cholinergic neuropeptides: key players in asthma. *Eur J Pharmacol* 2000;405:113-29.
 35. Reynolds PN, Holmes MD, Scicchitano R. Role of tachykinins in bronchial hyper-responsiveness. *Clin Exp Pharmacol Physiol* 1997;24:273-80.
 36. Advenier C, Joos G, Molimard M, Lagente V, Pauwels R. Role of tachykinins as contractile agonists of human airways in asthma. *Clin Exp Allergy* 1999;29:579-84.
 37. Widdicombe JG. Mediators and reflex bronchoconstriction. *Eur J Respir Dis Suppl* 1983;125:65-94.
 38. Joos GF, Germonpre PR, Pauwels RA. Neural mechanisms in asthma. *Clin Exp Allergy* 2000;30 Suppl 1:60-5.
 39. Kehrl JH. Heterotrimeric G protein signaling: roles in immune function and fine-tuning by RGS proteins. *Immunity* 1998;8:1-10.
 40. Girkontaite I, Missy K, Sakk V, Harenberg A, Tedford K, Poetzel T, et al. Lsc is required for marginal zone B cells, regulation of lymphocyte motility and immune responses. *Nat Immunol* 2001;2:855-62.
 41. Le LQ, Kabarowski JHS, Weng Z, Satterthwaite AB, Harvill ET, Jensen ER, et al. Mice lacking the orphan G protein-coupled receptor G2A develop a late-onset autoimmune syndrome. *Immunity* 2001;14:561-71.
 42. Stanners J, Kabouridis PS, McGuire KL, Tsoukas CD. Interaction between G proteins and tyrosine kinases upon T cell receptor-CD3-mediated signaling. *J Biol Chem* 1995;270:30635-42.
 43. Amatruda TT, Steele DA, Slepak VZ, Simon MI. Gα16, a G protein α subunit expressed in hematopoietic cells. *Proc Natl Acad Sci U S A* 1991;88:5587-91.
 44. Lippert E, Baltensperger K, Jacques Y, Hermouet S. Gα16 expression is up- and down-regulated following T-cell activation: disruption of this regulation impairs activation-induced cell responses. *FEBS Lett* 1997;417:292-6.
 45. Zhou J, Stanners J, Kabouridis P, Han H, Tsoukas CD. Inhibition of TCR/CD3-mediated signaling by a mutant of the hematopoietically expressed G16 GTP-binding protein. *Eur J Immunol* 1998;28:1645-55.
 46. Lowry WE, Huang X-Y. G protein βγ subunits act on the catalytic domain to stimulate Bruton's agammaglobulinemia tyrosine kinase. *J Biol Chem* 2002;277:1488-92.
 47. Bence K, Ma W, Kozasa T, Huang XY. Direct stimulation of Bruton's tyrosine kinase by Gq protein alpha-subunit. *Nature* 1997;389:296-9.
 48. Banu Y, Watanabe T. Augmentation of antigen receptor-mediated responses by histamine H1 receptor signaling. *J Exp Med* 1999;189:673-82.
 49. Jutel M, Watanabe T, Klunker S, Akdis M, Thomet OAR, Malolepszy J, et al. Histamine regulates T-cell and antibody responses by differential expression of H1 and H2 receptors. *Nature* 2001;413:420-5.
 50. Barnes PJ. Effects of β-agonists on inflammatory cells. *J Allergy Clin Immunol* 1999;104:S10-S17.
 51. Fujikura T, Shimosawa T, Yakuo I. Regulatory effect of histamine H1 receptor antagonist on the expression of messenger RNA encoding CC chemokines in the human nasal mucosa. *J Allergy Clin Immunol* 2001;107:123-8.
 52. Rothenberg ME, Zimmerman N, Mishra A, Brandt E, Birkenberger LA, Hogan SP, et al. Chemokines and chemokine receptors: their role in allergic airway disease. *J Clin Immunol* 1999;19:250-65.
 53. Devouassoux G, Metcalfe DD, Prussin C. Eotaxin potentiates antigen-dependent basophil IL-4 production. *J Immunol* 1999;163:107:2877-82.
 54. Mohanty JG, Raible DG, McDermott LJ, Pelleg A, Schulman ES. Effects of purine and pyrimidine nucleotides on intracellular Ca⁺⁺ in human eosinophils: activation of purinergic P2Y receptors. *J Allergy Clin Immunol* 2001;107:849-55.
 55. Marquardt D. Mast cell adenosine receptor characteristics and signaling. In: Griesmacher A, Chiba P, Muller MM, editors. *Purine and pyrimidine metabolism in man IX*. Oxford: Plenum Press; 1998. p. 79-82.
 56. Lynch KR, O'Neill GP, Liu Q, Im DS, Sawyer N, Metters KM, et al. Characterization of the human cysteinyl leukotriene CysLT1 receptor. *Nature* 1999;399:789-93.
 57. Yokomizo T, Izumi T, Chang K, Takawa Y, Shimizu T. A G-protein-coupled receptor for leukotriene B4 that mediates chemotaxis. *Nature* 1997;387:620-4.
 58. Yokomizo T, Kato K, Terawaki K, Izumi T, Shimizu T. A second leukotriene B4 receptor, BLT2: a new therapeutic target in inflammation and immunological disorders. *J Exp Med* 2000;192:421-31.
 59. Gaudreau R, LeGouill C, Metaoui S, Lemire S, Stankova J, Rola-Pleszczynski M. Signalling through the leukotriene B4 receptor involves both α and α16, but not αq or α11 G-protein subunits. *Biochem J* 1998;335:15-8.
 60. Bartemes KR, McKinney S, Gleich GR, Kita H. Endogenous platelet-activating factor is critically involved in effector functions of eosinophils stimulated with IL-5 or IgG. *J Immunol* 1999;162:2982-9.
 61. Druey KM. Bridging with GAPs: receptor communication through RGS proteins. *Science SKTE* 2001;104:RE14.
 62. Chaffin KE, Perlmutter RM. A pertussis toxin-sensitive process controls thymocyte emigration. *Eur J Immunol* 1991;21:2565-73.
 63. Chaffin KE, Beals CR, Wilkie TM, Forbush KA, Simon MI, Perelmutter RM. Dissection of thymocyte signaling pathways by in vivo expression of pertussis toxin ADP-ribosyltransferase. *EMBO J* 1990;3821-9.
 64. Luther SA, Cyster JG. Chemokines as regulators of T cell differentiation. *Nat Immunol* 2001;2:102-7.
 65. Hargreaves DC, Hyman PL, Lu TT, Ngo VN, Bidgol A, Suzuki G, et al. A coordinated change in chemokines responsiveness guides plasma cell movements. *J Exp Med* 2001;194:45-56.
 66. Gonzalo JA, Lloyd CM, Peled A, Delaney T, Coyle AJ, Gutierrez-Ramos JC. Critical involvement of the chemotactic axis CXCR4/SDF1α in the inflammatory component of allergic airway disease. *J Immunol* 2000;165:499-508.
 67. Kaburagi Y, Shimada Y, Nagaoka T, Hasegawa M, Takehara K, Sato S. Enhanced production of CC-chemokines (RANTES, MCP-1, MIP-1α, MIP-1β, and eotaxin) in patients with atopic dermatitis. *Arch Dermatol Res* 2001;293:350-5.
 68. Vestergaard C, Bang K, Gesser B, Yoneyama H, Matsushima K, Larsen CG. A Th2 chemokine, TARC, produced by keratinocytes may recruit CLA+CCR4+ lymphocytes into lesional atopic dermatitis skin. *J Invest Dermatol* 2000;115:640-6.
 69. Gao JL, Wynn TA, Chang Y, Lee EJ, Broxmeyer HE, Cooper S, et al. Impaired host defense, hematopoiesis, granulomatous inflammation and type 1-type 2 cytokine balance in mice lacking CC chemokine receptor 1. *J Exp Med* 1997;185:1959-68.
 70. Braun MC, Kelsall BL. Regulation of interleukin-12 production by G-protein-coupled receptors. *Microb Infect* 2001;3:99-107.
 71. Rudolph U, Finegold MJ, Rich SS, Harriman GR, Srinivasan Y, Brabet P,

- et al. Gi2a protein deficiency: a model of inflammatory bowel disease. *J Clin Immunol* 1995;15:101S-105S.
72. Rudolph U, Finegold MJ, Rich SS, Harriman GR, Srinivasan Y, Brabet P, et al. Ulcerative colitis and adenocarcinoma of the colon in $\text{G}\alpha\text{i}2$ -deficient mice. *Nat Genet* 1995;10:143-50.
 73. Hoernquist CE, Lu X, Rogers-Fani PM, Rudolph U, Shappell S, Birnbaumer L, et al. $\text{G}\alpha\text{i}2$ -deficient mice with colitis exhibit a local increase in memory $\text{CD}4^+$ T cells and proinflammatory $\text{T}_\text{H}1$ -type cytokines. *J Immunol* 1997;158:1068-77.
 74. Sasaki T, Irie-Sasaki J, Jones RG, Oliveira-dos-Santos AJ, Stanford WL, Bolon B, et al. Function of $\text{PI}3\text{K}\gamma$ in thymocyte development, T cell activation, and neutrophil migration. *Science* 2000;287:1040-6.
 75. Hirsch E, Katanaev VL, Garlanda C, Azzolino O, Pirola L, Silengo L, et al. Central role for G-protein-coupled phosphoinositide 3-kinase γ in inflammation. *Science* 2000;287:1049-53.
 76. Marinissen MJ, Gutkind JS. G-protein-coupled receptors and signaling networks: emerging paradigms. *Trends Pharmacol Sci* 2001;22:368-76.
 77. Finney PA, Belvisi MG, Donnelly LE, Chuang TT, Mak JCW, Scorer C, et al. Albuterol-induced downregulation of $\text{G}\alpha\text{s}$ accounts for pulmonary β_2 -adrenoceptor desensitization in vivo. *J Clin Invest* 2000;106:125-35.
 78. Ross EM, Wilkie TM. GTPase activating proteins for heterotrimeric G proteins: regulators of G protein signaling (RGS) and RGS-like proteins. *Ann Rev Biochem* 2000;69:795-827.
 79. Berman DM, Wilkie TM, Gilman AG. GAIP and RGS4 are GTPase activating proteins for the Gi subfamily of G protein alpha subunits. *Cell* 1996;86:445-52.
 80. Watson N, Linder ME, Druey KM, Kehrl JH, Blumer KJ. RGS family members: GTPase activating proteins for heterotrimeric G-protein α -subunits. *Nature* 1996;383:172-5.
 81. Zheng B, Ma YC, Ostrom RS, Lavoie C, Gill GN, Insel PA, et al. RGS-PX1, a GAP for $\text{G}\alpha\text{s}$ and sorting nexin in vesicular trafficking. *Science* 2001;294:1939-42.
 82. Hepler JR, Berman DM, Gilman AG, Kozasa T. RGS4 and GAIP are GTPase activating proteins for $\text{Gq}\alpha$ and block activation of phospholipase $\text{C}\beta$ by γ -thio-GTP- $\text{Gq}\alpha$. *Proc Natl Acad Sci U S A* 1997;94:428-32.
 83. Druey KM, Blumer KJ, Kang VH, Kehrl JH. Inhibition of G-protein-mediated MAP kinase activation by a new mammalian gene family. *Nature* 1996;379:742-6.
 84. Denecke B, Meyerdieks A, Bottger EC. RGS1 is expressed in monocytes and acts as a GTPase-activating protein for G-protein-coupled chemoattractant receptors. *J Biol Chem* 1999;274:26860-8.
 85. Cho H, Kozasa T, Takekoshi K, DeGuzenberg J, Kehrl JH. RGS14, a GTPase-activating protein for Gi-alpha, attenuates Gi-alpha and G13-alpha-mediated signaling pathways. *Mol Pharmacol* 2000;58:569-76.
 86. Scheschonka A, Dessauer CW, Sinnarajah S, Chidiac P, Shi CS, Kehrl JH. RGS3 is a GTPase-activating protein for Gi-alpha and Gq-alpha and a potent inhibitor of signaling by GTPase-deficient forms of Gq-alpha and G11-alpha. *Mol Pharmacol* 2000;58:719-28.
 87. Siderovski DP, Heximer SP, Forsdyke DR. A human gene encoding a putative helix-loop-helix phosphoprotein whose mRNA increases rapidly in cycloheximide-treated blood mononuclear cells. *DNA Cell Biol* 1994;13:125-47.
 88. Reif K, Cyster JG. RGS molecule expression in murine B-lymphocytes and ability to down-regulate chemotaxis to lymphoid chemokines. *J Immunol* 2000;164:4720-9.
 89. Beadling C, Druey KM, Richter G, Kehrl JH, Smith KA. Regulators of G protein signaling exhibit distinct patterns of gene expression and target G protein specificity in human lymphocytes. *J Immunol* 1999;162:2677-82.
 90. Oliveira-dos-Santos AJ, Matsumoto G, Snow BE, Bai D, Houston FP, Whishaw IQ, et al. Regulation of T cell activation, anxiety, and male aggression by RGS2. *Proc Natl Acad Sci U S A* 2000;97:12271-7.
 91. Pierce KL, Lefkowitz RJ. Classical and new roles of β -arrestins in the regulation of G-protein-coupled receptors. *Nat Rev Neurosci* 2001;2:727-33.
 92. Lefkowitz RJ. G protein coupled receptors III. New roles for receptor kinases and β -arrestins in receptor signaling and desensitization. *J Biol Chem* 1998;273:18677-80.
 93. Lombardi MS, Kavelaars, Schedlowski M, Bijlsma WJ, Okihara KL, VandePol M, et al. Decreased expression and activity of G-protein-coupled receptor kinases in peripheral blood mononuclear cells of patients with rheumatoid arthritis. *FASEB J* 1999;13:715-5.