The signaling cascades evoked by G protein-coupled receptors are a predominant mechanism of cellular communication. The regulators of G protein signaling (RGSs) comprise a family of proteins that attenuate G protein-mediated signal transduction. Here we report the characterization of RGS13, the smallest member of the RGS family, which has been cloned from human lung. RGS13 has been shown to possess GAP activity specifically toward Gαq and Gα12 or Gα13. Surprisingly, RGS13 inhibits cAMP generation elicited by stimulation of the β2-adrenergic receptor. These data suggest that RGS13 may regulate Gαq, Gα12, and Gα13-coupled signaling cascades.

G protein signaling is an effective mechanism of cellular communication during both physiological and pathological conditions (1–3). Regulators of G protein-signaling (RGS) proteins are a relatively new family of proteins that attenuate G protein-mediated pathways by acting as GTPase-activating proteins (GAPs) for Gα subunits (4). RGS binding stabilizes a Gα conformation that favors hydrolysis of GTP to GDP, which hastens the termination of active Gα (5). The second mechanism of G protein inhibition by RGS proteins is effector antagonism in which the RGS protein binds GTP-bound Gα and prevents Gα/effector interaction (6). RGS proteins share a common domain, the RGS box. This motif of 120 amino acids is highly conserved in all RGS family members and conveys the ability of RGSs to bind G proteins (7). Although many RGS proteins exhibit GAP activity toward members of the Gαq (8, 9) and Gα12 (10) families, only one RGS protein has been shown to possess GAP activity specifically toward Gαq (11). In addition to the classical RGS family, an additional group of proteins exists termed the RhoGEF RGSs (orRGS) (12, 13). These proteins, which contain a highly diverged RGS homology domain, act as GAPs specifically for Gα12/13 and also stimulate GTP binding by Rho family members.

Although all RGS proteins contain the conserved RGS box, they display significant variability in sequences outside of this region. For example, a cysteine string found in RGS-GAP and RGS20 is a site of palmitoylation, which may assist in membrane anchorage (14). The Gαq GAP RGS-PX1 also contains a Phox domain that may be involved in intracellular trafficking (11).

Unique motifs in certain RGSs may modify the G protein GTPase cycle or G protein effector stimulation in additional ways. RGS14, for example, contains a “GoLoco” motif that inhibits guanine nucleotide dissociation on Gα15. Most RGS proteins do not exhibit GAP activity toward Gα16; however, a short form of RGS3 (RGS3T) blocks calcitonin gene-related peptide-induced cAMP generation (16). More recently, it was shown that RGS2 directly inhibits some isoforms of adenylyl cyclase, independently of Gα (17), suggesting that RGS proteins can affect signaling through interactions with proteins downstream of Gα.

Here we report the characterization of RGS13, the smallest RGS found in mammalian tissues. Human RGS13 was cloned from lung cDNA and is expressed most prominently in immune tissues such as tonsil, thymus, lymph node, and spleen. As with other RGS proteins, recombinant RGS13 exhibits GAP activity toward Gαq family members and not Gαi or Gα12. RGS13 binds Gαq in the presence of AMF, implying that it acts as a GAP for Gαq as well. Transfected RGS13 blunts MAPK activation evoked by either Gαq or Gαi-coupled receptors. In addition, it blocks Gαq-QL-induced CRE-dependent transcription, consistent with a potential role for RGS13 as an effector antagonist of Gαq. RGS13 inhibits receptor-stimulated cAMP generation, suggesting that it regulates Gαq signaling despite its lack of Gαq GAP activity. Thus, RGS13 may regulate G protein-mediated processes in the lung and immune system.

EXPERIMENTAL PROCEDURES

Cell Lines and Plasmids—Stable transfectants of the m1 muscarinic receptor into Chinese hamster ovary (CHO) cells and m2 muscarinic receptor into A9L cells were the generous gift of Jurgen Wess (NIDDK, National Institutes of Health). Cells were grown in Dulbecco’s modified Eagle’s medium, 10% fetal calf serum, penicillin/streptomycin, glutamine (Invitrogen) (A9L and 293T) + NEAA (CHO). Plasmids directing expression of GαqQL, GαqQLGα12QL, or GαqQL were obtained from Silvio Gutkind (NIDCR, National Institutes of Health). CRE-Luciferase was purchased from Stratagene, and SRE-luciferase was the generous gift of Koza Kaibuchi (Nara Institute of Science and Technology, Ikoma, Nara, Japan).

Real-time TaqMan Polymerase Chain Reaction (PCR) for Quantification of cDNA—Real-time TaqMan PCR was performed using primers 5′-TCAATTCTGGATGCGATGGTA-3′ and 5′-TCTTGCTGAAACTGTCA-3′ in a TaqMan probe of 5′-ACCTATAAGAATTGCTTCACGGTGAGC-3′. The amount of RGS13 cDNA present was normalized to glyceraldehyde-3-phosphate dehydrogenase values and expressed as n-fold greater than the lowest detectable level using the
characteristic of cyclodrome. The identity of PCR products was verified by automated sequencing.

Cloning of RGS13—RGS13 was cloned from human lung cDNA (CLONTECH) using PCR with the following primers: 5’-ATAGCAGAGCACGCTGTGGATCT-3’ and 5’-TCAGAATGCATTGGAGACGCTAT-3’. The 0.6 kb PCR product was analyzed by agarose gel using a phophospecific Erk antibody (Santa Cruz Biotechnology). Protein detected by Erk-1 antibody (Santa Cruz Biotechnology) was used to examine the relative amount of protein.

Transcriptional Reporter Assays—3 × 10^5 293T cells were transfected in 6-well plates with 0.4 μg of SRE-luciferase or CREB-luciferase, 0.25 μg of LacZ, and 1.5 μg of GFP, GPP-RGS13, or GFP-RGS4 in the presence or absence of 0.2 μg of GoαLQ, GoαLQ (SRE-luc), or GoαQ (CREB-Luc) using SuperFect reagent (Qiagen). 24 h post-transfection cells were washed with phosphate-buffered saline and scraped into reporter lysis buffer (Promega). Luciferase substrate (Promega) or β-galactosidase substrate (Applied Biosystems) were added to cell lysates prior to determination of luciferase or β-galactosidase values in a Monolight lumimeter 3010 (Analytical Luminescence Laboratory).

Assay of Adenylyl Cyclase Activity—3 × 10^5 293T cells were transiently transfected with 0.2 μg of β₂-adrenergic receptor plus 1.5 μg of GPP or GPP-RGS13 using SuperFect (Qiagen). 24 h post-transfection, media was replaced with 1 μM isobutylmethylxanthine in the presence or absence of 1 μM isoproterenol for 10 min at 37 °C. Cold phosphate-buffered saline stopped the reaction. Cells were scraped into 4 mL EDTA, boiled to aggregate protein, and centrifuged at 14,000 × g for 10 min. Supernatants were assayed for cAMP using the Biotrak radioimmunoassay (Amersham Biosciences).

Results

Characterization of RGS13

RGS13 Is Enriched in Lung and Immune Tissues—To evaluate the expression of RGS13, we performed TaqMan analysis using cDNA from various tissues. We found RGS13 most abundantly in the tonsil followed by thymus, lymph node, lung, and spleen (Fig. 1A). Liver and pancreas exhibited intermediate levels of RGS13, whereas heart, skeletal muscle, kidney, and placenta expressed low levels. No RGS13 was detected in peripheral blood mononuclear cell, fetal liver, and brain. Thus, it appears that RGS13 is enriched in lymphoid tissues. To determine which cells within tonsil and other lymphoid compartments expressed RGS13, cDNAs from peripheral blood cells isolated with specific surface markers were assessed for RGS13 transcripts. Resting CD19+ (B cells) and CD14+ (monocytes) cells expressed the highest levels of RGS13, and similar to other RGS transcripts (1, 20, 21) RGS13 increased upon stimulation in CD19+ cells. Activated CD8+ (T cells) cells expressed very low levels of RGS13 (Fig. 1B).

Expression and Subcellular Localization of RGS13 Protein—To facilitate detection of RGS13 protein, we raised rabbit polyclonal antibodies against two distinct RGS13 peptides, one at the N terminus and a second at the C terminus. We utilized each antibody to reveal recombinant RGS proteins by immunoblotting. Both RGS13 peptide antibodies detected as little as 250 ng of recombinant RGS13 protein but failed to detect either recombinant RGS4 or RGS16 (Fig. 2A). We then utilized these antibodies to probe an immunoblot of detergent lysates from various human tissues including lung. However, we failed to detect expression of a specific band of the predicted molecular mass of RGS13 with either antibody under these conditions.

Several recent publications have addressed the issue of cellular localization of RGS proteins. To determine where exogenous RGS13 was expressed in mammalian cells, we constructed a vector directing expression of an RGS13-GFP fusion protein and transfected the plasmid into 293T cells. We lysed cells in hypertonic lysis buffer and fractionated membranes, cytosol, and nuclei by differential centrifugation. Equal
amounts of protein from each fraction were immunoblotted with anti-RGS13 antibodies. RGS13 was localized predominantly in the membrane and nuclear fractions with a very small amount in the cytosolic fraction (Fig. 2B).

RGS13 Is a Gαi GAP and Binds Gαq—To examine the function of RGS13 and its interaction with various G proteins we performed GAP assays. Recombinant His-tagged RGS13 was purified from bacteria by Ni²⁺ affinity chromatography and tested for GAP activity. RGS13 increased the rate of GTP hydrolysis by both Gαi and Gαo but not Gαs (Fig. 3, A–C). In addition, recombinant RGS13 bound purified Gαq only in the presence of AMP (Fig. 3D), suggesting that RGS13 possesses GAP activity toward Gαq as well.

RGS13 Inhibits Both Gαi and Gαq-mediated MAPK Activation—We evaluated the ability of RGS13 to block G protein-mediated signaling in intact cells by measuring G protein-coupled receptor-stimulated MAPK activation in the presence or absence of transfected RGS13. Carbachol stimulation of stably transfected Gαi-coupled m2 muscarinic receptors in A9L cells resulted in an 8-fold increase in MAPK activity as measured by phosphorylated Erk. Co-transfection of RGS13 blunted this response by almost 50% (Fig. 4A). Similarly, carbachol evoked an increase in phospho-Erk in 293T cells transiently transfected with the m1 muscarinic receptor, which is coupled to Gαq. This increase was also blocked by expression of RGS13, indicating that RGS13 inhibits Gαq-mediated MAPK activation as well (Fig. 4B).

RGS13 May Act as an Effector Antagonist for Gαq—Because some RGS proteins block the interaction between Gαq and its effector, PLCβ, we examined the ability of RGS13 to regulate transcription stimulated by GTPase-deficient G proteins. To assess signaling via Gαq, 293T cells were transiently trans-
fected with a CRE-Luciferase reporter gene in the presence or absence of Gq/H9251qQL. Co-transfection of RGS13 blocked Gq/H9251qQL-mediated CRE activation (Fig. 5A). However, RGS13 did not significantly inhibit transcription of a serum-response element.

**FIG. 3.** RGS13 interacts with Gαi, Gαo, and Gαq. GTPase assays were performed as described under "Experimental Procedures." A–C, hydrolysis of GTP by Gαi (A), Gαo (B), or Gαq (C) during a single catalytic turnover in the presence of recombinant RGS13 (dotted line), RGS16 (dashed line), or buffer (solid line). D, immunoblot of Gαq co-purified by His6-RGS proteins immobilized on Ni2+-agarose in the presence or absence of AMP.

**FIG. 4.** RGS13 attenuates signaling cascades initiated by stimulation of Gαi- or Gαq-coupled receptors. MAPK was activated by carbachol stimulation of muscarinic receptors in A9L-m2 cells (A) or 293T cells transfected with m1 muscarinic receptor (B). A phospho-Erk antibody was used to detect activated MAPK and was normalized to Erk-1 levels as revealed by an Erk-1/2 antibody. Cells were transiently transfected with GFP or GFP-RGS13. Bar graphs represent analysis of band intensity (mean ± S.E. of 4–6 independent experiments) by densitometry (*, p < 0.05, paired Student's t test).
luciferase reporter gene (SRE-Luciferase) induced by either 
GαqQL or Gα12QL (Fig. 5, B and C).

**RGS13 Inhibits Isoproterenol-induced cAMP Generation**—

Because it has been recently reported that certain RGS proteins inhibit some isoforms of adenylyl cyclase directly, we examined the ability of RGS13 to block cAMP production. 293T cells were transfected with the β2-adrenergic receptor in the presence of GFP or RGS13. Stimulation of these cells with isoproterenol evoked a 4-fold increase in cAMP. Co-transfection of RGS13 attenuated the increase in cAMP by approximately 50% (Fig. 6). Because RGS13 does not act as a Gαs GAP (Fig. 3 C), RGS13 may block this signaling pathway at the level of adenylyl cyclase.

**DISCUSSION**

This study represents the initial characterization of RGS13 expression and function. RGS13 is most abundant in human tonsil followed by thymus, lymph node, lung, and spleen with low levels or a lack of expression in various other tissues. Ectopically expressed GFP-RGS13 localizes in both membrane and nuclear fractions of 293T cells with a very small portion of RGS13 in the cytosol. Similar to other classical RGS proteins, RGS13 possesses GAP activity toward Gαi family members and inhibits signaling evoked by Gαi-coupled receptors. RGS13 binds to Gαq in the presence of AMF, suggesting that RGS13 is a GAP for Gαq. In addition to its GAP activity, RGS13 likely blocks Gαq signaling by effector antagonism as well because GTPase-deficient GαqQL-mediated CRE stimulation is attenuated by RGS13. RGS13 is not a GAP for Gαs, although it inhibits cAMP generation induced by stimulation of a Gαs-coupled receptor.

The expression pattern of RGS family members is highly variable and may contribute to physiological specificity of RGS proteins that share similar biochemical properties. RGS16 is expressed predominantly in the retina, pituitary, and liver (22–24). RGS18 is expressed in hematopoietic tissues and lung (25, 26), whereas RGS2 and RGS3 are ubiquitously expressed (20, 27). RGS13 expression is highest in tonsil (about 6.8 × 10^6 times greater than CD4+ cells). There is 10-fold less RGS13 in thymus, lymph node, lung, and spleen, with much lower levels of RGS13 detected in other tissues such as brain, which is a reservoir for many of the RGS family members (28, 29). In contrast to earlier reports describing RGS13 expression in brain (29, 30), we failed to detect any RGS13 in brain cDNA. This discrepancy could be explained by localization of RGS proteins within the brain; that is, certain areas of the brain

![Figure 5: RGS13 blocks transcription evoked by constitutively active Gαq, but not Gα12 or Gα13. 293T cells were transiently transfected with reporter gene constructs and GFP, GFP-RGS13, or GFP-RGS4. Transcription of CRE-Luciferase (A) or SRE-Luciferase (B and C) was measured in the presence or absence of co-transfected GαqQL (A), Gα12QL (B), or Gα13QL (C) as described under “Experimental Procedures.” (*, p < 0.01, repeated measures analysis of variance, Tukey-Kramer post-hoc test).

![Figure 6: RGS13 blocks cAMP generation. 293T cells were transiently transfected with β2-adrenergic receptor and GFP or GFP-RGS13. Cells were stimulated in the presence of 1 mM isobutylmethylxanthine with 5 μM isoproterenol for 10 min at 37 °C. Inset: intracellular cAMP was determined by radioimmunoassay. Bar graph represents mean ± S.E. of eight experiments (*, p < 0.05, paired Student’s t test).]
enriched with RGS13 may be underrepresented in total brain cDNA.

Using the RGS13 peptide antibodies, we evaluated endogenous RGS13 expression in human lung, spleen, liver, pancreas, kidney, heart, and brain (data not shown). We concluded that the peptide antibodies were able to detect recombinant RGS13 protein and not other closely related RGS proteins of similar size, the sensitivity of the peptide antibodies was insufficient to identify endogenous protein in tissue lysates by immunoblotting. To address this issue, we have initiated generation of knockout mice in which a LacZ reporter gene is placed under control of the RGS13 promoter, which should allow us to better define the anatomical and developmental expression of RGS13.

Transfected RGS13 localizes in the cell membrane and the nucleus. Several RGS proteins have been localized to the cell membrane (31) and have undergone post-translational modifications such as palmitoylation, which may assist in membrane anchorage (32, 33). RGS13 lacks several of the N-terminal residues that adopt an α-helical conformation in closely related RGSs such as RGS4, 5, and 16 and facilitate direct interaction with anionic membrane phospholipids (34, 35). It will be interesting to determine whether palmitoylation of RGS13 is required for its membrane localization or whether another mechanism of membrane targeting is responsible. It has been reported that RGS2 and RGS10 are localized in the nucleus (36). We show here that transfected RGS13 is also located in the nucleus, although the functional significance of nuclear RGS expression has yet to be determined.

RGS13 functions similarly to many RGS proteins in that it bears GAP activity toward members of the Goα family and likely has GAP activity toward Goβγ as well. RGS13 may be an effector antagonist for Goα as demonstrated by its ability to block GoαQL-mediated CRE activation. Although one could postulate that the capacity of RGS13 to block cAMP generation may have influenced the CRE-Luciferase reporter assay, the lack of significant decrease in either basal cAMP levels or basal CRE-Luciferase activity suggests that this mechanism would not account for the inhibition of GoαQL-stimulated CRE-Luciferase by RGS13.

RGS13 blunted isoproterenol-evoked increases in cAMP but exhibited no GAP activity toward Goβγ. There are several possible explanations for this result. An interaction between RGS9 and a phosphodiesterase has been reported (37). RGS13 may enhance cAMP metabolism by stimulating a phosphodiesterase, resulting in a net decrease in cAMP concentration. In that case one would expect to see decreased basal levels of cAMP, which we failed to detect. A truncated form of RGS3 (RGS3T) was shown to decrease receptor-mediated cAMP generation without decreasing basal cAMP levels (16). The authors suggested that it was unlikely that RGS3T blocked adenylyl cyclase directly. Given the similarity in our results, RGS13 and RGS3T may be acting via the same uncharacterized mechanism. Another possibility is that like RGS2 (17) RGS13 may block adenylyl cyclase directly. It has been demonstrated that RGS1, RGS2, and RGS3 (but not RGS4 or RGS5) block GTPγS/odorant-mediated cAMP generation in olfactory epithelium membranes. In addition, RGS2 was shown to block forskolin-evoked increases in cAMP (17), indicating that some RGS proteins regulate certain isoforms of adenylyl cyclase directly. Interestingly, RGS2 failed to attenuate cAMP production induced by stimulation of endogenous adenylyl cyclase in HEK-293 cells (17). In contrast we found that RGS13 inhibited cAMP generation in these cells, suggesting that RGS2 and RGS13 may regulate different isoforms of adenylyl cyclase. Given that the only identified domain in RGS13 is the RGS box, it will be of interest to examine whether the RGS box is the region that confers the ability of RGS proteins to inhibit CAMP production. If so, why then do RGS4 and RGS5 lack the ability to regulate this process?

Functional analysis of RGS13 reported here was derived from cells transfected with GFP-RGS13. We utilized GFP-RGS13 only after several failed attempts to express RGS13 in untagged or His-tagged forms. Although we used the GFP vector or other GFP-tagged RGS proteins as controls, the difficulty in expressing untagged RGS13 is of interest. Future experiments are required to determine whether the difficulty in detecting untagged RGS13 is a result of such anomalies as unstable transcription or translation. This explanation seems unlikely because we could easily detect in vitro-translated untagged RGS13 in rabbit reticulocyte lysates (data not shown). Alternatively, RGS13 may require a binding partner or modification for stable expression in mammalian cells. An example of this type of protein instability is Gβδ, which requires expression of GαS9 in the brain (18). These studies might also explain the difficulty in detecting endogenous levels of RGS13 by immunoblotting.

Through the characterization of RGS13 we have added another piece to the puzzle of the RGS family. Unlike many RGS proteins RGS13 has no identified domain other than the RGS box, but because of its high expression in the immune system and lung and its ability to block Goα, Goβγ, and cAMP generation, the biological niche of RGS13 might be to regulate specific G protein-dependent signal transduction pathways in these regions. The physiological relevance of RGS13 and other RGS proteins is slowly being evaluated through the use of targeted gene disruption and expression of transgenes in mice. Through these lines of experimentation we hope to better understand the function of RGS13 and its relevance to health and disease.

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REFERENCES

1. Beadling, C., Druey, K. M., Richter, G., Kehrl, J. H., and Smith, K. A. (1999) J. Immunol. 162, 2677–2682
2. Johnson, E. N., and Druey, K. M. (2002) J. Allergy Clin. Immunol., in press
3. Gilman, A. G. (1987) Annu. Rev. Biochem. 56, 615–649
4. Ross, E. M., and Wilkie, T. M. (2000) Annu. Rev. Biochem. 69, 795–827
5. Tesmer, J. J., Berman, D. M., Gilman, A. G., and Sprang, S. R. (1997) Cell 89, 251–261
6. Hepler, J. R., Berman, D. M., Gilman, A. G., and Kurzasa, T. (1997) Proc. Natl. Acad. Sci. U. S. A. 94, 428–432
7. De Vries, L., Zheng, B., Fischer, T., Elenko, E., and Farquhar, M. G. (2000) Annu. Rev. Pharmacol. Toxicol. 40, 235–271
8. Berman, D. M., Wilkie, T. M., and Gilman, A. G. (1996) Cell 86, 445–452
9. Watson, N., Linder, M. E., Druey, K. M., Kehrl, J. H., and Blumberg, K. J. (1996) Nature 383, 172–175
10. Mukhopadhyay, S., and Ross, E. M. (1999) Proc. Natl. Acad. Sci. U. S. A. 96, 9539–9544
11. Zheng, B., Ma, Y. C., Ostrom, R. S., Lavie, C., Gill, G. N., Insel, P. A., Huang, X. Y., and Farquhar, M. G. (2001) Science 294, 1399–1424
12. Chen, Z., Wells, C. D., Sternweis, P. C., and Sprang, S. R. (2001) J. Biol. Chem. 276, 432–434
13. Longenecker, K. L., Lewis, M. E., Chikumi, H., Gutkind, J. S., and Derewenda, M. I. (2000) J. Mol. Biol. 298, 746–748
14. Dennis, J. C., Morrison, E. E., Vodyanoy, V., and Kehrl, J. H. (2001) Annu. Rev. Immunol. 19, 891–921
15. Siderovski, D. P., Strockbine, B., and Behe, C. I. (1999) Mol. Biol. Cell 10, 403–415
16. Chatterjee, T. K., Resnick, A. K., and Fisher, R. A. (1997) J. Biol. Chem. 272, 15481–15487
17. Reif, K., and Cyster, J. G. (2000) J. Immunol. 164, 4720–4729
18. Chen, C., Zheng, B., Han, J., and Lin, S. C. (1997) J. Biol. Chem. 272, 8679–8685
