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Regulators of G Protein Signaling Exhibit Distinct Patterns of Gene Expression and Target G Protein Specificity in Human Lymphocytes

Carol Beadling,* Kirk M. Druey,† Gunther Richter,‡ John H. Kehrl,‡ and Kendall A. Smith*³

The newly recognized regulators of G protein signaling (RGS) attenuate heterotrimeric G protein signaling pathways. We have cloned an IL-2-induced gene from human T cells, cytokine-responsive gene 1, which encodes a member of the RGS family, RGS16. The RGS16 protein binds $G_{i\alpha}$ and $G_{q\alpha}$ proteins present in T cells, and inhibits $G_{i\alpha}$- and $G_{q\alpha}$-mediated signaling pathways. By comparison, the mitogen-induced RGS2 inhibits $G_{i\alpha}$ but not $G_{q\alpha}$ signaling. Moreover, the two RGS genes exhibit marked differences in expression patterns. The IL-2-induced expression of the RGS16 gene in T cells is suppressed by elevated cAMP, whereas the RGS2 gene shows a reciprocal pattern of regulation by these stimuli. Because the mitogen and cytokine receptors that trigger expression of RGS16 and RGS16 in T cells do not activate heterotrimeric G proteins, these RGS proteins and the G proteins that they regulate may play a heretofore unrecognized role in T cell functional responses to Ag and cytokine activation. The Journal of Immunology, 1999, 162: 2677–2682.

Heterotrimeric G proteins that are comprised of $\alpha$, $\beta$, and $\gamma$ subunits couple with members of a family of receptor molecules that have seven transmembrane-spanning segments and are termed G protein-coupled receptors (GPCRs)⁴. Thus far, the GPCRs expressed on lymphocytes and their respective G proteins have not been found to play major roles in lymphocyte activation, which is regulated by Ag receptors and cytokine receptors, neither of which couple with G proteins. However, several lines of evidence suggest that both TCR-, as well as IL-2R-driven events may be intertwined with heterotrimeric G proteins and their signaling pathways. For example, both cytokine production and T cell proliferation are inhibited by PGE₁ and PGE₂ (1). These mediators are produced by activated macrophages during immune responses and elicit their effects through specific GPCRs. In addition, T cells from $G_{i\alpha}$ knockout mice show a marked increase in TCR-induced production of IL-2, TNF, and IFN-γ relative to wild-type controls, and TCR-induced proliferation is increased in the $G_{i\alpha}$ knockout mice (2), indicating that heterotrimeric G proteins may well function to modulate both TCR- and IL-2R-stimulated signaling pathways.

Recently, a possible mechanism whereby Ag or cytokine receptor signalng might interface with heterotrimeric G proteins was suggested by the discovery and characterization of a new family of molecules termed regulators of G protein signaling (RGS; reviewed in Ref. 3). RGS proteins inhibit heterotrimeric G protein-mediated signaling by accelerating the rate of GTP hydrolysis by the $G_{i\alpha}$ subunits (4). Thus, these RGS proteins are GTPase-activating proteins (GAPs) that are specific for the heterotrimeric G proteins and function in a fashion similar to the GAPs that are well known to regulate the smaller monomeric G proteins such as p21ras. Heterotrimeric G protein complexes are activated upon GTP binding to the $G_{s\alpha}$ subunit, with dissociation of the $G_{s\alpha}$-GTP from $G_{b\gamma}$. The intrinsic GTPase activity of $G_{s\alpha}$ drives GTP hydrolysis to GDP and $G_{s\alpha}$-GDP reassociation with $G_{b\gamma}$. Thus, like the monomeric G protein GAPs, RGS-GAP activity favors accumulation of the $G_{s\alpha}$ subunits in a GDP-bound inactive state, promoting the reassociation of $G_{s\alpha}$ with $G_{b\gamma}$ subunits, thereby attenuating both the $G_{s\alpha}$ and $G_{b\gamma}$ effector pathways. There are four families of $G_{s\alpha}$ subunits, $G_{s1}$, $G_{s2}$, $G_{s3}$, and $G_{s4}$; the $G_{s1}$ and $G_{s4}$ appear to be the primary targets of RGS proteins (3).

To date, >20 members of the RGS family have been described (3, 5, 6). Notably, two of the first mammalian RGS genes identified, RGS1 (BL34/IR20) and RGS2 (G0S8), were isolated by virtue of their mitogen-induced expression in lymphocytes (7–9). RGS1 expression is induced in B cells by phorbol ester, staphylococcal protein A, surface Ig, and by IL-4, cAMP, or platelet-activating factors as well (5, 7, 10). In comparison, the expression of the RGS2 gene that was originally termed $G_{i\alpha}$ switch gene 8 is induced upon Con A stimulation of the $G_{i\alpha}$-$G_{i\gamma}$ transition in T cells (9, 11). Con A has been shown to bind the TCR/CD3 complex (12–14) and the T cell mitogenic response to Con A is blocked by Fab of an anti-CD3 mAb (12). Thus, it is likely that the induction of RGS2 expression is mediated at least in part through the TCR/CD3 complex, although the involvement of additional accessory signals cannot be ruled out. Therefore, stimuli that induce RGS gene expression, such as Ags, mitogens, and cytokines, and stimul

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4 Abbreviations used in this paper: GPCR, G protein-coupled receptors; RGS, regulators of G protein signaling; GAP, GTPase-activating protein; MAP, mitogen-activated protein; CREB, cAMP response-element-binding protein; $\beta$-gal, $\beta$-galactosidase; HA, hemagglutinin; ERKs, extracellular signal-regulated kinase; CR1, cytokine-responsive gene 1; $\beta_{2}$-m, $\beta_{2}$-microglobulin.
which activate heterotrimeric G proteins (e.g., PG and chemo- kines) may modulate one another via RGS proteins.

Previously, we described the isolation of IL-2-induced intermedi- ate/early genes from human T cells (15). We report here the character- ization of the cytokine responsive gene 1 (CR1), which is a member of the RGS family. This gene was also cloned by Buck- binder et al. (16) and has been designated RGS16 (17). The RGS16 gene exhibits a broad pattern of constitutive expression in every tissue examined with highest expression in the retina (16, 17). In vitro assays have demonstrated that rRGS16 protein binds with high affinity (Kd, ~35 nM) to transducin, the retina-specific GTPase involved in visual signal transduction. Assays with reconstitu- ted rod outer segment membranes showed that RGS16 acceler- ates transducin GTPase activity 10-fold (18). Thus, a role for RGS16 in visual signal transduction is clearly implicated. Potential functions of this gene product in lymphocytes have not been addressed.

As the GTPase-promoting activity of the RGS proteins is cons- titutive, the regulation of expression of the RGS genes may well be an important means to control RGS function. Therefore, the present studies were undertaken to identify the stimuli that regulate expression of the RGS2 and RGS16 genes in human T cells and to identify the Gs subunits targeted by the respective gene products. Our results, detailed in this report, demonstrate that RGS function is regulated by the inherent target specificity of individual RGS proteins toward distinct Gs subunits and also by differential regulation of expression of the RGS genes. Moreover, these results lend further support to the notion that in T cells, non-G protein-coupled receptors such as the IL-2R may use RGS proteins to influence heterotrimeric G protein signaling pathways.

Materials and Methods

Cell culture

PBCs were isolated from the venous blood of healthy donors by Ficoll- Hypaque (Pharmacia, Uppsala, Sweden) density centrifugation. Lympho- cytes were cultured in RPMI 1640 (Life Technologies, Gaithersburg, MD) supplemented with 10% FCS (Gemini BioProducts, Calabasas, CA) and antibiotics and were stimulated with a 1:500 dilution of an anti-CD3 mAb (OKT3; Ortho Pharmaceuticals, Raritan, NJ). After 3 days of OKT3 stim- ulation, cells were washed and replaced in culture for an additional 11 days in the presence of 500 pM IL-2 (Takeda, Osaka, Japan). Cells were washed out of IL-2 and replaced in culture for 36 h, then restimulated with 50 ng/ml phorbol-12,13-dibutyrate (Calbiochem, San Diego, CA) to induce high-affinity IL-2R expression. Cells were washed of phorbol-12,13-dibu- tyrate for 12 h before restimulation. This enabled generation of a synchro- nized-T cell population which was arrested in early G1 and comprised of >90% CD8+ T cells (19). The phorbol ester treatment does not affect the expression of RGS2 (11) or RGS16 (15). The U937 and the 293 human leukemia cell lines (20) were maintained in RPMI 1640 with 10% FCS and antibiotics.

Cellular proliferation, Northern blot analysis, and RT-PCR analysis

IL-2R+ T cells were restimulated with 500 pM IL-2 alone or in combina- tion with 0.5 mM dibutyryl-cAMP (Sigma, St. Louis, MO) or 0.5 mM sodium butyrate (Sigma). PGE1 or PGE2 (Sigma) were used at a final

concentration with 0.5 mM dibutyryl-cAMP (Sigma, St. Louis, MO) or 0.5 mM

influence heterotrimeric G protein signaling pathways.

Our results, detailed in this report, demonstrate that RGS function is regulated by the inherent target specificity of individual RGS proteins toward distinct Gs subunits and also by differential regulation of expression of the RGS genes. Moreover, these results lend further support to the notion that in T cells, non-G protein-coupled receptors such as the IL-2R may use RGS proteins to influence heterotrimeric G protein signaling pathways.

Preparation of rRGS16 protein

Hexa-histidine-tagged RGS16 protein (His6RGS16) was generated by cloning a cDNA fragment encoding the 202-amino acid RGS16 open read- ing frame into the pRSET vector (Invitrogen, Carlsbad, CA). The RGS16 construct was expressed in BL21/DE3/pLysS bacteria (Novagen, Madison, WI), and protein expression was induced by incubation with 1 mM isopro- pionyl β-thio-galactopyranoside for 3 h at 37°C. Soluble recombinant protein was purified by binding to Ni2+ NTαA resin (Qiagen, Chatsworth, CA) according to manufacturer’s instructions and eluted with a 50 mM to 300 mM imidazole gradient. Aliquots of eluted fractions were analyzed by SDS-PAGE and Coomassie Blue staining, and fractions containing RGS16 (>90% pure) were used for further assays.

Gs-binding assays

Affinity purification of endogenous G proteins with rRGS16 protein was performed as follows. Jurkat cells (5 × 108) were lysed in buffer containing 50 mM HEPEs (pH 8.0), 300 mM NaCl, 1 mM DTT, 6 mM MgCl2, and 1% Triton X-100. Cell lysates were then activated with GDP (30 μM) or GDP plus 30 μM AlF4− and 100 mM NaF for 30 min at 30°C. Lysates were then incubated for 1 h with 4°C with 20 μg His6RGS16 and 60 μl of a 50% slurry of Ni2+ -NTαA beads (Qiagen). After one wash with buffer A (same as above buffer, but with 0.025% C12E10 detergent (Sigma) substituted for Triton X-100), bound proteins were eluted with Laemmli buffer and boiled for 5 min. After separation by SDS-PAGE and transfer to nitrocellulose filter, blots were probed with antisera against Gαi1 (a gift of Allen Spie- gel, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health), Gαo (DuPont, Boston, MA), or Gαs (Santa Cruz Biotechnologies, Santa Cruz, CA).

Cellular signaling assays

To monitor the Gαi-linked CXCR1 pathway, mitogen-activated protein (MAP) kinase activation was measured in HEK 293, stably expressing CXCR1. Cells were transiently cotransfected with 2 μg of hemagglutinin (HA-) extracellular signal-regulated kinase 1 (ERK1) and 5 μg of RGS expression plasmid by the calcium phosphate method and subsequently stimulated for 3 min with IL-8 (50 ng/ml). MAP kinase activity was measured as previously described (5).

To measure the activity of the Gαo-linked m1 muscarinic pathway, a CAMP-response element-binding protein- (CREB-) β-galactosidase (β-gal) reporter gene (kindly provided by Roger Cone, Oregon Health Sciences University, Portland, OR) was used. Briefly, 1 μg of CMBE-β-gal, 1 μg of m1 receptor expression plasmid (the kind gift of J. Silvio Gutkind, National Institute of Dental Research, National Institutes of Health) and 4 μg of RGS expression plasmids were cotransfected into 293 T cells by the calcium phosphate method. A constitutively active Gq mutant, Gq/Q209L, was also assayed by cotransfection with the CREB-β-gal reporter. Cells were serum-starved for 24 h, and after 48 h m1 receptor-transfected cells were stimulated for 6 h with 1 mM carbachol (Sigma). Cell extracts were prepared and 10 μl of supernatant was incubated in diluted β-gal substrate (Galacton; Tropix, Bedford, MA), and the luminescence measured using a Monolight 3010 Luminometer (Analytical Luminescence Laboratories,
San Diego, CA). Each point was standardized by measuring the protein concentrations of the lysates.

To determine expression of RGS plasmids, each construct (RGS2, RGS4, RGS16) was epitope tagged with an HA peptide. The remaining supernatants from each assay were immunoprecipitated with HA Ab (BAbCo, Richmond, CA), separated by SDS-PAGE, and then immunoblotted again with the HA Ab.

Results

CR1 homologies with RGS proteins

CR1 was identified and cloned in a screen for IL-2-induced immediate/early genes in human T cells (15). The full-length 2.4-kb CR1 cDNA encodes a predicted open reading frame of 202 amino acids. Sequence alignments indicate that CR1 is a member of the RGS family, identical to RGS16 (GenBank accession number U70426) (16, 17). This human RGS gene was also cloned by Buckbinder et al. (16), who termed it RGS14. However, the designation RGS14 was assigned earlier to a partial cDNA described by Koelle and Horvitz (6) in their original description of the RGS family. Chen et al. (22) cloned the murine homologue, RGS16, which was previously called RGS-r by virtue of its high expression in the retina (23). The human gene has also been referred to as RGS-r (18). To clarify the nomenclature, the Human Genome Organization/Genome Data Base Nomenclature Committee has designated this gene RGS16 (17).

RGS16 contains a core RGS domain between amino acids 62 and 180, which is highly conserved among RGS proteins from yeast, Caenorhabditis elegans, and mammals (24). Among human RGS proteins, RGS16 is most similar to RGS3 (the C-terminal 175 amino acids, GenBank U27655), RGS4 (U27768), RGS2 (L13463), RGS1 (S59049), and RGS-GAIP (EMBL X91809). The overall identities of these proteins with RGS16 range from 38 to 49%. All show the highest degree of sequence conservation within the RGS domain. The murine RGS16 protein (U72881) is 86% identical to the human, and the rat homologue (AA817864) shows 86% identity to the human RGS16 protein (16, 18, 22, 23).

Reciprocal effect of elevated intracellular cAMP on expression of RGS16 and RGS2 genes

Although both RGS16 and RGS2 were originally cloned from T lymphocytes, mitogen stimulation induces the expression of RGS2 during the G0/G1 cell cycle transition (9), whereas IL-2R activation induces RGS16 during the G1-S phase transition (15). To analyze further the expression patterns of these RGS genes in T cells, we chose to examine the effect of elevated intracellular cAMP, which inhibits both TCR and IL-2R signaling (25), and is well known to be a second messenger activated by GPCRs. As shown in Fig. 1A, IL-2-induced DNA synthesis in IL-2R+ human peripheral blood-derived T cells, as monitored by [3H]thymidine uptake, was completely inhibited by the membrane permeant cAMP analogue, dibutyryl-cAMP, but not by sodium butyrate. Northern blot analysis revealed marked differences in the patterns of RGS16 and RGS2 expression (Fig. 1B). RGS16 transcripts were expressed at very low levels in G1-synchronized IL-2R+ cells, but exhibited significant induction after 2 h of IL-2 stimulation. Dibutyryl-cAMP completely blocked the IL-2 stimulatory effect, whereas the sodium butyrate did not. In contrast, RGS2 transcripts were already present at high levels as a result of completing the G0-G1 transition. However, IL-2 stimulation suppressed this RGS2 expression, and elevation of intracellular cAMP reversed the IL-2 inhibition. Thus, the RGS16 and RGS2 genes exhibit reciprocal patterns of expression in response to IL-2 stimulation, and elevated intracellular cAMP also modulates the expression of the two genes in a reciprocal fashion.

As the pharmacologic elevation of intracellular cAMP concentrations influenced both RGS16 and RGS2 expression, the effects of the physiologic elevation of cAMP by PGE1 and PGE2 were examined in the U937 monocyte cell line that expresses high levels of PGE receptors. As shown in Fig. 1C, both PGE1 and PGE2 inhibited cellular proliferation by ~50%, which is consistent with previous observations (1). Just as in the case of dibutyryl-cAMP, both PGE1 and PGE2 inhibited RGS16 expression, whereas RGS2 expression was increased (Fig. 1D). However, there was no effect on β2m expression. Thus, the RGS16 and RGS2 genes exhibit distinct patterns of expression in response to physiologic GPCRs that elevate intracellular cAMP.

Binding of RGS16 to endogenous G proteins in T cells

The differences in expression patterns of the RGS16 and RGS2 genes suggested different functions of the respective gene products. In this regard, the RGS2 protein is known to bind and activate the GTPase activity of Gqα subunits, but not Giα subunits (26). To identify the target Gα protein specificity of RGS16 and to determine the relevant interactions that occur between RGS16 and Gα proteins in T lymphocytes, we examined the binding of RGS16 protein to Gα subunits present in Jurkat T leukemic cells. Previous analyses of other RGS proteins demonstrated little or no RGS
Single turnover GTPase assays indicated that RGS16 accelerates binding to “inactive” GDP-bound Gα subunits, but high-affinity binding to Gα subunits complexed with GDP-AlF4 that mimics the transition state of GTP hydrolysis (24). Therefore, lysates of the Jurkat T cell line were treated with excess GDP or activated with GDP+AlF4− and then incubated with recombinant hexa-histidine-tagged (His6) RGS16 immobilized on Ni2+/NTA beads. After washing away unbound proteins and separating eluted proteins by SDS-PAGE, the blots were probed for various Gα subunits. No G proteins from these lysates bound to beads alone or to beads coupled to an irrelevant hexahistidine-tagged protein (data not shown). In addition, as shown in Fig. 2, little or no binding of endogenous Gα proteins was seen when the lysates were treated with GDP alone. In contrast, strong binding of endogenous Gα1–3 and Gαq to immobilized RGS16 was seen when the lysates were treated with AlF4−, consistent with the binding activity of other RGS proteins (4, 27, 28), and with observations of RGS16 binding to in vitro translated Gα1 (29). Thus, in contrast to RGS2, RGS16 binds to both Gαi and Gαq subunits.

Differential inhibition of Gαi- and Gαq-mediated cellular signaling by RGS2 and RGS16

Single turnover GTPase assays indicated that RGS16 accelerates the GTPase activities of Gαi1, Gαi2, and Gαi3 subunits in vitro by ~10-fold, whereas RGS2 could not serve as a GAP for any of the Gαi subunits in solution assays, consistent with its Gαi-binding characteristics (data not shown). Because Gαq could not be tested in vitro for technical reasons, we reconstituted in vivo signaling pathways mediated by Gαi and Gαq in HEK 293 cells to determine whether the differences in binding and in the activities of RGS2 and RGS16 proteins toward Gαi and Gαq subunits observed in vitro were reflected in differential regulation of intracellular G-coupled signaling pathways in vivo. As a control, RGS4 was used, because this RGS protein has been well characterized as an inhibitor of both Gαi- and Gαq-mediated signaling (4). To assess the effects of RGS protein expression on a Gαq-linked pathway, we measured MAP kinase activity in 293 cells expressing CXCR1 after stimulation with the chemokine IL-8. An 8-fold increase in MAP kinase activity was observed in stimulated versus nonstimulated cells, and cells transfected with either RGS2 or RGS4 were inhibited by only ~25%. By comparison, cells expressing RGS16 consistently revealed an inhibition of IL-8-induced MAP kinase activation by >60% (Fig. 3A). Similar results were observed when 293 cells stably transfected with CCR5 and transiently transfected with RGS proteins were stimulated with RANTES (data not shown).

The activity of a Gαq-linked pathway was measured by the activity of a CREB-β-gal reporter gene in cells stimulated by the m1 muscarinic receptor, and in cells expressing a constitutively active, GTPase-deficient Gαq (GαqQ290L) (30). The CREB-β-gal construct reflects the activation of CREB resulting from an increase in intracellular Ca2+ or cAMP (31). In cells expressing the m1 receptor, we observed an ~12-fold increase in reporter gene expression in cells stimulated with carbachol. Coexpression of RGS2, RGS4, or RGS16 substantially inhibited this pathway, with the strength of the activity ranked as RGS2 ≫ RGS16 > RGS4 (Fig. 4A). These results confirm and extend those previously described for RGS16 (16) Similar results were observed in cells expressing constitutively activated Gαq (Q290L): both RGS4 and RGS16 inhibited reporter gene expression only moderately, whereas in cells that expressed RGS2, Gαq activity was actually below basal levels (Fig. 4B). In all signaling assays, variable expression of transfected RGS proteins could not account for the differences in inhibition observed, as levels were similar in HA immunoprecipitates (for MAP kinase assays; Fig. 3B), or in equivalent amounts of total cellular lysates blotted with HA Ab (all other assays; data not shown). These results are consistent with the recently reported specificity of RGS2 for attenuating Gαq (26), and underscore the functional heterogeneity of RGS proteins that may be expressed in the same cell.

**FIGURE 2**. RGS16 binds Gαi and Gαq proteins in T cells. Jurkat cell lysates treated with GDP or AlF4− as indicated were incubated with His6 RGS16 immobilized on Ni2+/NTA beads. Bound proteins were separated by SDS-PAGE and detected by Western blot analysis with antisera against Gαi1–3, Gαi4, and Gαq. Lane 1 represents one-tenth of the starting Jurkat cell lysate as a relative control for the amount of Gα protein present.

**FIGURE 3**. RGS16, but not RGS2, inhibits Gαi-mediated cellular signaling pathways. A, HEK 293 cells, stably transfected with a CXCR1 receptor, were transiently transfected with HA-ERK1 and RGS2, RGS4, or RGS16 expression plasmids as indicated. IL-8-induced MAP kinase activity was monitored by incorporation of [32P]-ATP into myelin basic protein incubated with HA-immunoprecipitated lysates. Bar graph values represent the mean ± SEM fold increase in activity over unstimulated cells. Values represent at least three independent experiments. B, Expression of HA epitope-tagged RGS proteins was detected by anti-HA immunoprecipitation and Western blot analysis with anti-HA antisera.
The inhibition of Gq- and Gi-mediated CREB b ...ector families. As its designation implies, Gia acts to inhibit the activity of adenylate cyclase, thereby countering the generation of elevated intracellular cAMP. When viewed from the IL-2 signaled events, i.e., G1 progression to the S phase of the cell cycle, it is logical that the IL-2R should attenuate GPCRs that result in competitive or counteractive signaling pathways. Therefore, Gi activation and resultant Ca2+ flux and PKC activation would necessarily generate biochemical events that may counteract IL-2R signaling. The attenuation of Gia by RGS16 is more difficult to understand, given that elevated intracellular cAMP inhibits IL-2-promoted G1 progression (25, 32). IL-2-induced RGS16 expression in T cells is transient, peaking at 2–4 h and declining rapidly thereafter (15). Therefore, it is possible that the turnover of RGS16 in early G1 promotes late G1 progression.

The pharmacologic effect of dibutyryl cAMP and the physiologic effect of PGE1 and PGE2 on RGS2 and RGS16 expression are of interest in that the regulation of RGS gene expression by cAMP provides for an interconnection between Gs and the other Gs subunits. Both PGE1 and PGE2 trigger activation of adenylate cyclase and elevation of intracellular cAMP, through the Gs-coupled EP2 and EP4 receptors (33, 34). Recently, elevation of cAMP has been shown to decrease RGS4 mRNA levels, and augment RGS2 levels, in PC12 cells. This effect could be achieved by pharmacologic elevation of cAMP with forskolin or cAMP analogues, and also by ligand stimulation of the Gs-coupled adenosine receptor A2a (35). To date, no RGS protein has been identified that acts as a GAP toward Gia, so that the regulation of RGS genes by Gs-coupled stimuli may serve to modulate signaling by other GPCRs, rather than in an autoregulatory loop. Clearly, there are several different levels of control, whereby both GPCRs and non-GPCRs can regulate one another via the RGS proteins.

The most well-characterized GPCRs in lymphocytes are those that bind chemokines. In vitro analyses (36), as well as in vivo studies with transplanted pertussis toxin-treated cells (37) and with transgenic mice expressing the catalytic subunit of pertussis toxin under the control of the lck promoter (38, 39), have demonstrated that lymphocyte migration is sensitive to the toxin. Pertussis toxin ADP-ribosylates and inactivates Gia subunits, indicating that the chemotactic response requires Gia. As Gia subunits are targets of RGS16, it would be predicted that IL-2-triggered RGS16 expression could serve to attenuate the chemotactic signaling pathway. Notably, IL-2 has been reported to inhibit the chemotactic response of CD4+ and CD8+ T cells to both IL-8 and RANTES (40). This inhibition may well be effected by the IL-2-mediated induction of RGS16.

Focusing only on Gs signaling gives an incomplete treatment of the potential effects of the RGS proteins, in that there are 6 β-chain gene products and 12 γ-chain gene products, in addition to the >20 α-chain gene products that comprise the four main functional...
groups of $G_\alpha$ proteins. Thus far, effector proteins of the $G_\alpha$ complex include phospholipases, adenylate cyclases, ion channels, G-protein-coupled kinases, phosphoinositol 3-kinases, and the non-receptor tyrosine protein kinases, Btk and Tsk (3). Accordingly, to understand the role that RGS proteins play in T cell activation, proliferation, and differentiation, it will be necessary to identify exactly which G protein $\alpha$, $\beta$, and $\gamma$ subunits are expressed in T cells and to delineate which signaling pathways are coupled with both the $G_\alpha$ and the $G_\beta\gamma$ subunits. In this regard, the discovery that the RGS2 and RGS16 genes are regulated by mitogen and IL-2, respectively, provides for a starting point for such an experimental dissection.

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