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The impact of RGS and other G-protein regulatory proteins on $G\alpha_i$-mediated signaling in immunity

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Abstract

Leukocyte chemoattractant receptors are members of the G-protein coupled receptor (GPCR) family. Signaling downstream of these receptors directs the localization, positioning and homeostatic trafficking of leukocytes; as well as their recruitment to, and their retention at, inflammatory sites. Ligand induced changes in the molecular conformation of chemoattractant receptors results in the engagement of heterotrimeric G-proteins, which promotes α subunits to undergo GTP/GDP exchange. This results in the functionally release of βγ subunits from the heterotrimers, thereby activating downstream effector molecules, which initiate leukocyte polarization, gradient sensing, and directional migration. Pertussis toxin ADP ribosylates $G\alpha_i$ subunits and prevents chemoattractant receptors from triggering $G\alpha_i$ nucleotide exchange. The use of pertussis toxin revealed the essential importance of $G\alpha_i$ subunit nucleotide exchange for chemoattractant receptor signaling. More recent studies have identified a range of regulatory mechanisms that target these receptors and their associated heterotrimeric G-proteins, thereby helping to control the magnitude, kinetics, and duration of signaling. A failure in these regulatory pathways can lead to impaired receptor signaling and immunopathology. The analysis of mice with targeted deletions of $G\alpha_i$ isoforms as well as some of these G-protein regulatory proteins is providing insights into their roles in chemoattractant receptor signaling.

Graphical Abstract

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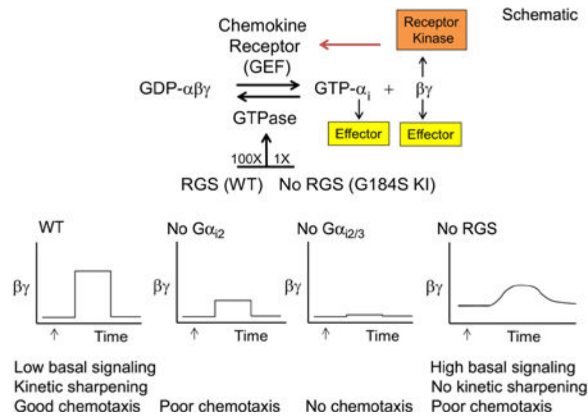

Keywords

G-protein; RGS proteins; chemokine receptors; sphingosine 1-phosphate; cell trafficking

1. Introduction

Upon ligand binding chemoattractant GPCRs undergoes conformational rearrangements that change their interaction with signal transducer proteins (1–3). The major proximal signal transducers of an activated GPCR are their cognate heterotrimeric G-proteins, although ligand activated GPCRs engage other proteins including G-protein receptor kinases (GRKs) and β-arrestins (4–6). Chemoattractant receptors predominately couple to the Gi family of heterotrimeric G-proteins as their signaling is sensitive to treatment with pertussis toxin, which ADP ribosylates a cysteine residue near the C-termini of α_i subunit (7,8). This inhibits $G\alpha_i$ from undergoing GPCR-mediated nucleotide exchange. The GTP/GDP binding status of $G\alpha_i$ controls its interaction with the other members of the trimeric G protein, the Gβγ dimer (2,9). Composed of one of 5 β and one of 12 γ subunits, the Gβγ dimers, once assembled, are inseparable. Receptor activation and $G\alpha_i$ subunit nucleotide exchange causes the $G\alpha_i$ subunit and Gβγ subunits to functionally dissociate. GTP bound $G\alpha_i$ and the freed Gβγ can then engage downstream effector molecules. However, it is the Gβγ effectors that are the most crucial for gradient sensing and directional cell migration (10). The activated Gα subunits remain only transiently GTP bound as they possess an intrinsic GTPase activity, which converts their bound GTP to GDP. This encourages the reassembly of the heterotrimeric G-protein terminating signaling, but also allows the G-protein to engage other activated receptors, thereby initiating another round of signaling. The intrinsic GTPase activity of Gα subunits can be greatly enhanced by GTPase activating proteins termed GAPs. Most $G\alpha_i$ GAPs contain a Regulator of G-protein signaling (RGS) domain (11–13). Other G-protein regulatory proteins include those that have a G-protein regulatory (GPR) domain, also called a GoLoco motif, which acts as a guanine nucleotide dissociation inhibitor (GDI) much like Gβγ (14–16). Both RGS and GPR domain containing proteins impact G_i signaling. This review will focus predominately on the $G\alpha_i$ proteins and their regulators in the context of chemokine and chemoattractant receptor signaling.

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2. Gi proteins

The heterotrimeric G proteins are divided into four classes; Gi, Gq, G12/13, and Gs, based on the amino acid sequences of their α subunit (2). The expression levels of the various Gα subunits in murine leukocytes based on mRNA sequencing are shown in Table 1. The "inhibitory class" of heterotrimeric G-proteins were originally named based on the ability of Gi/o proteins to inhibit adenylyl cyclase activity. Based on amino acid sequence homology the Gi family now includes not only Gi/o, but also Gt, Ggust, and Gz. The Gi/o subfamily includes 4 members, three Gi proteins and Go. As Go (*Gnao*) is poorly expressed in murine leukocytes it will not be further discussed. Of the three $G\alpha_i$ isoforms, murine leukocytes predominately express $G\alpha_{i2}$ and $G\alpha_{i3}$ with *Gnai2* mRNA transcripts 3–10 fold more abundant (16). $G\alpha_{i1}$ is nearly undetectable although low levels of *Gnai1* mRNA transcripts have been found in mouse TCRγδ cells and Tregs by RNA sequencing (http://www.immgen.org/databrowser/index.html). Each $G\alpha_i$ isoform can theoretically pair with 60 different Gβγ dimers. However, not all of the theoretical dimers are likely to be assembled *in vivo* and many of the subunits have a rather limited expression profile. For example, murine lymphocytes predominately express mRNA transcripts that encode 2 of the 5 Gβ subunits; $G\beta_1$, $G\beta_2$, and 4 of 12 Gγ subunits; $G\gamma_2$, $G\gamma_7$, $G\gamma_8$, and $G\gamma_{10}$ (16). The signaling specificities of the various Gβγ dimers, in general and for immune cell function specifically, are largely unknown.

While this review is focused on $G\alpha_i$ proteins in GPCR and chemoattractant receptor signaling there is an increasing appreciation that $G\alpha_i$ proteins as well other Gα subunits have functional roles at other intracellular sites. $G\alpha_i$ proteins have been localized in the endoplasmic reticulum, centrosomes, and midbody as well as associated with microtubules (17,18). In model organism and in mammals $G\alpha_i$ proteins have been ascribed functional roles in asymmetric cell divisions. Whether non-canonical $G\alpha_i$ signaling contributes to lymphocyte function remains largely unexplored (16).

2.1. $G\alpha_i$ signaling

The release of GTP-$G\alpha_i$ lowers intracellular cAMP levels by reducing the activity of certain adenylyl cyclase isoforms. While lowering cAMP levels may promote leukocyte chemotaxis, previous studies have not found an essential role for $G\alpha_i$ in chemokine directed cell migration (10). However several recent studies have identified new $G\alpha_{i2}$ interacting proteins. One such protein is Homer 3, which acts as a scaffold that spatially organizes actin assembly to support neutrophil polarity and motility downstream of GPCR activation in mice (19). However, in contrast to traditional G-protein effectors Homer 3 binds both the GTP and GDP bound forms of $G\alpha_{i2}$. While GTP-$G\alpha_i$ likely has a limited role in chemokine receptor signaling, freed Gβγ subunits are known to activate several signaling pathways needed for gradient sensing and chemotaxis (20–23). An early consequence of the engagement of chemokine receptors is the acquisition of a polarized shape with the cell adopting a leading edge and a trailing uropod. The activation of small GTPases such as Rac, Cdc42, RhoA, and Rap1; many of which are activated by GEFs downstream of Gβγ, are crucial for these early morphological changes and eventually for actin reorganization and cell motility (24). Other direct effector of Gi released Gβγ include PI3Kγ (phosphoinositide

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3-kinase γ), which plays a crucial role in inflammatory and allergic processes (25–27) ; phospholipase C β2 and β3 (28,29), which cause increases in intracellular calcium; GRK2, which phosphorylates certain chemokine receptors promoting β-arrestin recruitment (30); and mTor, which has been ascribed a role in chemotaxis (31–33).

2.2. Pertussis toxin

The recognition that pertussis toxin ADP ribosylates the $G\alpha_i$ subunits and inhibits GPCR triggered nucleotide exchange has proven extremely useful for studying GPCRs that signal though Gi (7,8,34–37). While similarly affecting all the $G\alpha_i$ isoforms, the experimental usage of pertussis toxin strongly implicated $G\alpha_i$ signaling in leukocyte trafficking, blood vessel egress, and the organization of lymphoid organ architecture. *Ex vivo* treatment of human and murine sdneutrophils, monocytes, and lymphocytes with pertussis toxin nearly eliminated *in vitro* chemotaxis and inhibited chemokine induced firm adhesion. The adoptive transfer of *ex vivo* treated lymphocytes into mice prevented the transferred lymphocytes from entering the splenic while pulp or entering into lymph nodes (38). *Ex vivo* treated lymphocytes observed in the high endothelial venules (HEVs) of adoptively transferred mice failed to firmly adhere to high endothelial venules. HEVs are specialized sites in the lymph node microvasculature where blood borne lymphocytes are captured and cross into the lymph node parenchyma. Transgenic expression of pertussis toxin in mice interfered with mature thymocyte reverse transmigration from the thymus medulla into the blood (39). As a consequence mature thymocytes accumulated in the thymus. However, there are some caveats in interpreting results from pertussis toxin experiments (40). Pertussis toxin has an AB5 configuration, one active subunit termed S1 and five binding subunits. The B oligomer binds to glycoconjugate molecules present on most mammalian cell types and the exotoxin enters the cell by endocytosis. As the B subunit oligomer can impact intracellular signaling pathways independent of the enzymatic activity of the S1 subunit care must be taken in not attributing these effects to the inhibition of $G\alpha_i$ signaling. The development of a double mutant PT9K/129G molecule that retains normal cellular binding activity, but lacks enzymatic activity has allowed the discrimination between the ADP-ribosylation activity of the S1 subunit, and signaling initiated by the B subunit oligomer (40). In addition, while $G\alpha_i$ is the major target for pertussis toxin mediated ADP ribosylation, other substrates are also modified. Yet when properly utilized to study GPCR signaling, pertussis toxin sensitivity strongly implicates the involvement of $G\alpha_i$ proteins. Another limitation of pertussis toxin usages is its inability to discriminate between the different pertussis toxin sensitive G-proteins. The development of $G\alpha_i$ isoform specific knock-out mice avoided some of these issues and provided additional insights into the importance of Gi signaling in lymphocytes and other leukocytes.

2.3. Gnai2$^{-/-}$ mice

Despite a compensatory increase in $G\alpha_{i3}$ expression *Gnai2*$^{-/-}$ mice have numerous immune phenotypes (41). These mice develop a Th1-mediated inflammatory colitis reminiscent of human ulcerative colitis, whose penetrance depends upon the genetic background of the mice. A C57BL/6 background confers some resistance to colitis development. C57BL/6 *Gnai2*$^{-/-}$ mice maintained in ventilated cages at the NIH under do not show evidence of colitis (J. Kehrl, unpublished observation), however a recent report indicates that C57BL/6

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kept under conventional conditions do develop colitis (42). *Gnai2* deficient mice often lack inguinal and other peripheral lymph nodes; have few, if any, Peyer's patches; and have an accelerated thymic involution (43–45).

2.3.1 B cells from Gnai2$^{-/-}$ mice—Immune phenotyping of these mice revealed a reduced numbers of splenic marginal zone and T2 transitional B cells; reduced peritoneal B-1a, but increased peritoneal B-1b B cells; and decreased Peyer's patch B cells (43). Indicating that the B cell phenotypes were lymphocyte intrinsic *Gnai2*$^{-/-}$ bone marrow reconstituted *Rag2*$^{-/-}$ mice exhibited a similar set of phenotypes (43). Isolated B cells from the *Gnai2*$^{-/-}$ mice have diminished responses to chemokines and sphingosine 1-phosphate (44). Consistent with defective chemokine receptor signaling *Gnai2*$^{-/-}$ B cells adoptively transferred into wild type mice adhered poorly to high endothelial venules (HEVs), entered lymph nodes inefficiently, tended to accumulate around the HEVs, and those cells that gained access to the lymph node moved much less vigorously than wild type B cells (44).

2.3.2 T cells from Gnai2$^{-/-}$ mice—*Gnai2*$^{-/-}$ mice have thymuses with normal or slightly increased number of thymocytes compared to wild type mice (41). Yet thymic progenitors from them home less efficiently to the thymus than do wild type cells (46). Thymocytes subset analysis (C57BL/6 or 129SvEv prior to colitis) revealed a reduction in double positive (DP) cells with an increase in single positive (SP) cells. Both an increased rate of differentiation of DP to SP cells along with a decreased egress of the SP cells have been proposed to explain the expansion of SP cells in the thymus (47,48). C57BL/6 *Gnai2*$^{-/-}$ mice maintained at the National Institutes Health have a modest reduction in DP cells, with a significant increase in mature SP cells (unpublished data). CD4 as well as CD8 T cells purified from spleens of these mice responded poorly to chemokines (49). The *Gnai2*$^{-/-}$ T cells have severe defects in chemokine-induced intracellular calcium mobilization, chemotaxis, and homing, whereas *Gnai2*$^{+/-}$ T cells exhibit modest defects. Intravital imaging revealed that the *Gnai2*$^{-/-}$ CD4 T accumulated at the lymph node cortical ridge failing to normally access the T cell zone. These cells also lacked the customary amoeboid-like cell movements of normal CD4 T cells (49).

2.3.2 Neutrophils from Gnai2$^{-/-}$ mice—*Gnai2*$^{-/-}$ neutrophils also exhibited defective response to chemokines and chemoattractants (50). They arrested poorly on inflamed endothelium and did not accumulate at inflammatory sites. While wild type neutrophils re-inforce a wound site the *Gnai2*$^{-/-}$ neutrophils did not being excluded from the central neutral cluster (51).

2.3.3 Macrophages from Gnai2$^{-/-}$ mice—*Gnai2*$^{-/-}$ macrophages exhibited similar defects to those of the *Gnai2*$^{-/-}$ neutrophils did (52). They were poorly recruited to the peritoneum following thioglycollate-induced peritonitis and to the lung following lipopolysaccharide (LPS)-triggered inflammation. Furthermore, knockdown of *Gnai2* mRNA expression decreased both the chemokine induced migration and motility of RAW 264.7 cells, which was rescued by restoring $G\alpha_{i2}$ expression (52).

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2.4. Gnai3$^{-/-}$ mice

In contrast to the *Gnai2*$^{-/-}$ mice few immune related phenotypes have been reported in the *Gnai3*$^{-/-}$ mice although a recent report showed that $G\alpha_{i3}$ functions along with $G\alpha_{i1}$ in macrophage TLR4 signaling (53). Lymphocytes, neutrophils, and macrophages all express significant levels of $G\alpha_{i3}$ yet, chemokine responses are intact in the above cells purified from *Gnai3*$^{-/-}$ mice (10,52,54,55). There is a report that *Gnai3*$^{-/-}$ effector T cells actually respond better to certain chemokines than do wild type cells and another that early *Gnai3*$^{-/-}$ thymocyte progenitors do not populate the thymus as well as wild type progenitors (46,56). The C57BL/6 *Gnai3*$^{-/-}$ mice maintained at the NIH have normal numbers of thymocytes, slightly reduced numbers of splenocytes, and normal numbers of lymph node cells (unpublished observation). Thymocyte subsets are unperturbed, but splenic and Peyer's patch B220^{+} cells are slightly reduced. The report alluded to above (53) showed that TLR4 signaling led to formation of a complex that contained $G\alpha_{i3,}$ CD14, and Gab1 (growth factor receptor binding 2 (Grb2)-associated binding protein 1), which promoted Akt activation. A deficiency in $G\alpha_{i3}$ reduced LPS-induced TLR4 endocytosis and interfered with phosphorylation of interferon regulatory factor 3. Silencing *Gnai3*/*Gnai1* expression in bone marrow-derived macrophages caused a M2-like phenotype with reduced LPS-induced cytokine production. The macrophages used in this study were prepared from mice on a mixed 129 background. Isoform specific immunoblotting indicated that they expressed a significant level of $G\alpha_{i1}$. This differs from C57BL/6 macrophages, which express little or no *Gnai1* mRNA or $G\alpha_{i1}$ protein.

2.5. Gnai2$^{fl/fl}$ mb1-cre mice

Mice lacking $G\alpha_{i2}$ expression in the B cell lineage were created by crossing mice carrying a floxed *Gnai2* allele to mice expressing the *cre* recombinase from the *mb1* locus (55). These mice delete a portion of the *Gnai2* coding sequence at the pre-B cell stage of development and they confirmed the B cell intrinsic origin of most of the B cell phenotypes found in the *Gnai2*$^{-/-}$ mice. The B cell specific loss of $G\alpha_{i2}$ resulted in a severe B cell trafficking problem. Similar to the *Gnai2*$^{-/-}$ mice marginal zone B cell development and B cell responses to chemokines were both impaired.

2.6. Gnai3$^{-/-}$Gnai2$^{fl/fl}$mb1-cre mice

Gnai2$^{-/-}$*Gnai3*$^{-/-}$ mice are not viable (57); however, conditional deletion of *Gnai2* on a *Gnai3*$^{-/-}$ background is possible. *Gnai3*$^{-/-}$*Gnai2*$^{fl/fl}$*mb1-cre mice* lack $G\alpha_{i2}$ and $G\alpha_{i3}$ expression in B cells (55). In these conditional double knock-out mice mucosal sites, splenic marginal zones, and lymph nodes essentially lacked B cells. There was a 50–60% reduction in splenic B cells with few if any marginal zone B cells. The spleen, lymph nodes, and the gastrointestinal tract lacked any organized B cell compartments. Purified splenic B cells were refractory to chemokine stimulation. The mice developed a hyper-IgM like syndrome having an elevated serum IgM level and with reductions in the other serum isotypes. The loss of $G\alpha_{i2}$ and $G\alpha_{i3}$ in mouse B cells led to a complete failure in their responsiveness to chemoattractants.

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3. G-protein regulatory proteins

3.1 Ric-8A

Ric-8 was identified in *C. elegans* based on its role in asymmetric cell divisions during early development (58–60). A human homologue, Ric-8A, was shown to recruit a signaling complex to the cell cortex that helped orient the mitotic spindle in response to spatial clues (61). Targeting *ric8* in mice caused early embryonic lethality, however, derived *ric8*$^{-/-}$ embryonic cell lines had pleiotropic G protein signaling defects and major losses of $G\alpha_{i1/2}$, $G\alpha_q$, and $G\alpha_{13}$ proteins due to rapid protein degradation (62–64). These later observations revealed that Ric-8A functioned as a molecular chaperone to target newly synthesized $G\alpha_i$, $G\alpha_q$, and $G\alpha_{12/13}$ proteins to cellular membranes. Conditionally targeting *ric8* in murine hematopoietic cells using the *cre* recombinase expressed in either all hematopoietic cells or only in murine B cells also led to severe reductions of Gα proteins with major decreases in $G\alpha_{i2/i3}$, $G\alpha_q$, and $G\alpha_{13}$ proteins (65). In the hematopoietic cell specific deletion, the mice had a major reduction of platelets, which likely accounted for their shortened lifespan. The B lymphocyte specific deletion did not impact mouse viability, but led to a severe B cell immune phenotype (65). While bone marrow B cell lymphopoiesis was not, splenic marginal zone B cell development was severely compromised. There was also a marked reduction of B cells numbers in the spleen, lymph nodes, and at other peripheral sites. Not surprisingly B cells from these mice responded poorly to chemoattractants. B cell trafficking and the *in situ* positioning of B cells was impaired, the lymphoid architecture disorganized, and antibody responses suboptimal. A photograph and schematic of the intravital imaging set-up to analyze the behavior of wild type and Ric-8A deficient B cells is shown (Figure 2). The wild type and Ric-8A deficient B cells were fluorescently labeled and adoptively transferred into a wild type mouse 1 day prior to imaging. The cells were imaged in the inguinal lymph node of an anesthetized mouse. The Ric-8A deficient B cells moved slower and poorly accessed the lymph node follicle when compared to wild type B cells. The major loss of $G\alpha_i$ proteins in the B cells of these mice likely accounts for the majority of the phenotypes. Suggesting that Ric-8A has additional functions in B cells independent of its role as a $G\alpha_i$ chaperone, the number of asymmetric cell divisions detected *in vitro* in activated B cell and among purified germinal center B cells were reduced.

3.2. AGS proteins

A functional screen in yeast for proteins that activated G-protein signaling in the absence of GPCRs led to the identification of what were termed AGS (Activators of G-protein signaling) proteins (14,66). They were initially divided into three functional groups: group I, guanine nucleotide exchange factors or GEFs of which there was a solitary member AGS1; group II, guanine nucleotide dissociation inhibitors, GDIs, of which there were four members AGS3-6; and group III, Gβγ interactors of which there were 5 members AGS2 & AGS7–10. Some of these AGS proteins are well expressed in immune cells and this review will mention several. Schematics of the domain structure of the known group II AGS proteins are shown (Figure 3). These proteins possess from 1 to 4 GoLoco motifs, which bind GDP, but not GTP bound $G\alpha_i$ much like Gβγ subunits. AGS3-5 (GPSM1-3) are briefly discussed below. A more detailed discussion of these and other AGS proteins can be found in other reviews (14,15,67,68). RGS12 and RGS14 are included in the group II AGS

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proteins as they each possess a GoLoco motif. However, since they also have an RGS domain they are considered in the section on RGS proteins. Not mention below are PCP2 and RAP1GAP1. PCP2 is expressed exclusively in cerebellar Purkinje cells and retinal bipolar neurons, where it impacts Gi/o signaling (69). The GoLoco motif in Rap1GAP1 is functional and may regulate the GAP activity of the protein (70). Ric-8A mentioned above is a group 1 AGS protein as it can trigger $G\alpha_i$ nucleotide exchange although not when Gα is associated with Gβγ (71). Recent evidence indicates that some group II members when bound to Gαi can sense agonist-induced conformational changes in GPCRs. This would suggest that they have a more complicated relationship with GPCR signaling than originally thought (72).

3.2.1. GPSM1/AGS3—The official gene name for AGS3 is *Gpsm1*, which encodes for a protein with 7 tetratricopeptide (TPR) repeats and 4 GoLoco motifs. The protein is most often referred to as AGS3. As mentioned above the GoLoco motifs can interact with GDP bound $G\alpha_i$. Multiple TPR motifs (3–16) form a TPR repeat, which acts as a scaffold to mediate protein–protein interactions. *Gpsm1* mRNA transcripts are broadly expressed found in most cell type including hematopoietic cells (16). Its expression is elevated in murine macrophages following LPS activation (73). Several studies have implicated AGS3 in chemokine receptor signaling. *Gpsm1*$^{-/-}$ mouse dendritic cells and lymphocytes exhibit suboptimal responds to chemokines in chemotaxis, calcium mobilization, and effector protein activation assays (74). Conversely, AGS3 may have a positive role in neutrophil chemotaxis. At the neutrophil leading edge GDP-bound $G\alpha_i$ accumulates where it recruits an adaptor molecule Inscuteable (Insc) along with AGS3 and the Par3-aPKC polarity complex (75). Neutrophils lacking Insc poorly stabilize leading edge pseudopods, which can be restored by the addition of wild-type Insc protein, but not by a mutant protein incapable of binding AGS3 (75). In RBL-2H3-CXCR2 cells, a mast cell line overexpressing CXCR2, exposure to a CXCR2 ligand resulted in the rapid formation of a GRK6/AGS3/$G\alpha_{i2}$ complex (76). Overexpression of AGS3 in these cells inhibited CXCR2 signaling while knock-down enhanced signaling without affecting receptor internalization (76).

3.2.2. GPSM2/AGS5/LGN—AGS5/GPSM2/LGN also encodes for a protein with 7 TPR and 4 GoLoco motifs. *Gpsm2* mRNA transcripts are well expressed in murine cycling pre-B cells, germinal center B cells, and in double positive thymocyte blasts (16). The protein is most commonly referred to as LGN and it has been shown to have an essential roles in asymmetric cell divisions in several different cell types. The binding of $G\alpha_i$ to the C-terminal GoLoco motif releases the auto-inhibited form of LGN allowing the N-terminal TPR motifs to interact with mInsc and NuMA, 2 proteins involved in cell division (77,78). Whether LGN has a role in asymmetric cell divisions in lymphocytes is unknown.

3.2.3. GPSM3/AGS4—GPSM3/AGS4 encodes for a protein that has 3 GoLoco motifs, but it lacks the TPR motifs. *Gpsm3* mRNA has a selective tissue distribution being well expressed in immune system cells with its highest level of expression in neutrophils (16). Besides interacting with GDP-$G\alpha_i$ via its GPR motif, AGS4 also interacts with Gβ independent of any interaction with Gα or Gγ. The interaction with Gβ may act to stabilize to Gβ prior to its binding of Gγ. Genome-wide association studies have shown that single

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nucleotide polymorphisms in *GPSM3* are associated with chronic inflammatory diseases (16).

3.3. RGS proteins

RGS proteins are a diverse family of proteins that are defined by the presence of a Regulator of G-protein Signaling (RGS) domain (11,13,79). The mammalian RGS family has more than 30 members, if those proteins with domains that exhibit weak homology to the RGS domain are included. The overall domain structure of the classical RGS proteins is shown (Figure 3). RGS family members can act as GAPs for $G\alpha_i$ and/or $G\alpha_q$ and several can attenuate Gs or G12/13 signaling. Most RGS domains bind with high affinity to $G\alpha_i$ subunits undergoing GTP hydrolysis. This interaction accelerates the intrinsic GTPase activity of the Gα subunit as much as a 100 fold. By shortening the duration that Gα is GTP bound, RGS proteins reduce the duration that GTP-Gα can interact with effectors. Since GDP-Gα can rebind freed Gβγ RGS proteins also limit Gβγ signaling. As such RGS proteins set a threshold for Gα-protein activation and sharpen the decay of signaling responses. Initially, RGS proteins were predicted to decrease steady-state GPCR agonist sensitivity, but this was not found. Explaining this observation several studies showed that RGS proteins not only increased the rate of G-protein deactivation, but also the rate of G-protein activation. The enhanced onset of signaling was sufficient to maintain agonist sensitivity and response amplitude. Several models have been proposed to account for this. An attractive model is termed “kinetic scaffolding” or “spatial focusing” (80,81). This model proposes that G-protein activation becomes saturated near spatially constrained GPCRs. This leads to GTP hydrolysis, rather than GDP release, becoming the rate limiting step in the G-protein cycle. Available RGS proteins by accelerating Gα GTP hydrolysis would promote heterotrimers re-assembly and provide additional heterotrimeric G-proteins for GPCR-activation. In the absence of RGS proteins, slow diffusion and collision events can not provide the needed heterotrimeric G-proteins for rapid and sustained activation of the signaling pathway. A prediction of this model is that loss of all $G\alpha_i$/RGS protein interactions would lead to a substantial disruption of $G\alpha_i$-mediated GPCR signaling (see section 3.3.11). The graphical abstract for this review illustrates the impact of the loss of $G\alpha_i$ proteins and RGS/$G\alpha_i$ protein interactions on the output of an idealized GPCR signaling pathway. While many RGS proteins predominately consist of an RGS domain, other RGS proteins contain multifunctional motifs and domains. These help mediate cross talk between GPCR-dependent and -independent signaling pathways. Furthermore, cell/tissue-specific expression patterns of many of the RGS proteins and their ability to interact with other signaling molecules helps restrict their signaling specificity. Many RGS proteins are expressed in human and murine immune cells (see Table 2, murine results shown) and there is considerable evidence that they influence immune cell function. The R4 RGS proteins are particularly well expressed and many of them impact chemokine receptor signaling by acting as GAPs for $G\alpha_i$ proteins (16). The R4 family includes RGS1-5, RGS8, RGS13, RGS16, RGS18, and RGS21 (82). In humans and mice, 9 of the 10 genes encoding the R4 RGS proteins are located in groups of two or more genes on chromosome 1. The sole exception is RGS3, which is located on chromosome 9 in humans and chromosome 4 in mice. Interestingly, the R4 RGS genes are closely linked to an MHC paralogon on chromosome 1 (83). The RGS proteins known to have roles in G-protein signaling in

leukocytes are discussed below and table 3 outlines the impact of RGS protein gene targeting in mice.

3. 3.1. RGS1—RGS1 is an R4 RGS protein. It and RGS2 were the founding members of the RGS protein family (84,85). Alterations in the *RGS1* gene are associated with celiac disease, multiple sclerosis, type I diabetes, and various types of human cancer (68,86). RGS1 is highly expressed in regulatory T cells, intraepithelial lymphocytes, activated B cells, NK-T cells, basophils, eosinophils, mast cells, dendritic cells, and activated macrophages (16). Expression is much higher in T cells from human gut versus peripheral blood, and that this can be exaggerated in intestinal inflammation (87). RGS1 expression is upregulated in many cell types by exposure to hypoxia or interferons (68,88). Reduced RGS1 levels have been shown to enhance T cell, B cell, dendritic, and macrophage responsiveness to chemokines (87,89,90). The roles of RGS1 in mast cell, eosinophil, and basophil biology are largely unknown, but the particularly high levels of expression of *RGS1* in these cell types suggests that is functionally important for their biology.

3.3.2. RGS2—RGS2 is an R4 RGS protein and its coding region is located in close proximity to *Rgs1* and *Rgs13* on chromosome 1. In immune system cell types *Rgs2* mRNA expression is very similar to that of *Rgs1* mRNA with a couple of exceptions. *Rgs2* is highly expressed in neutrophils, while *Rgs1* is not, and in contrast to *Rgs1*, *Rgs2* expression is not interferon inducible (16). Despite the similar expression pattern to *Rgs1*, *Rgs2* has not been linked to diseases of the immune system, but rather to cardiovascular and central nervous system dysfunction (91,92). However, analysis of mice lacking *Rgs2* expression did reveal a role for RGS2 in T cell proliferation and anti-viral immune responses (93). RGS2 differs from other RGS proteins as its GAP activity is largely restricted to $G\alpha_q$, showing little activity toward $G\alpha_i$. RGS2 also interacts with Gαs and adenylyl cyclase (AC), suppressing Gαs signaling pathways independently of its GAP activity (94–96). Thus, RGS2 is unlikely to impact chemokine receptor signaling, but to influence signaling through Gq- and Gs-linked receptors.

3.3.3. RGS3—With the exception of RGS3 the other R4 RGS protein family members are predominately an RGS domain with an n-terminal and c-terminal extension. There are several RGS3 splice variants that encode for proteins with additional domains. One variant termed PDZ-RGS3 has N terminal PDZ domain, which can bind type B ephrins, an ATP/GTP-binding site, and a proline-rich region of unknown function (97,98). A loss of PDZ-RGS3 in mice caused an early cell cycle exit and precocious differentiation of neural progenitor cells located in the developing cerebral cortex, a phenotype similar to that observed in the ephrin-B1 knockout mice. This phenotype was linked to dysregulated CXCR4 signaling (99). The original human RGS3 cDNA lacked the coding region for the PDZ domain and the predicted protein was termed RGS3L to distinguish it from a smaller RGS3 protein designated RGS3S (84). Both RGS3L and RGS3S are expressed in immune cells while PDZ-RGS3 is more prominent in neurons. Among immune cell types murine NK, NK-T, T cells, and neutrophils best express RGS3 mRNA transcripts (16). In a mouse model of asthma RGS3 affected the recruitment of inflammatory T cells and modulation of RGS3 levels affected the chemotaxis of a human T cell line (100).

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3.3.4. RGS10—Among the RGS proteins RGS10 is particularly well expressed in cells of the immune system (101). Based on homology of its RGS domain to those of other RGS proteins it is considered a member of the R12 subfamily. High levels of expression are present in murine dendritic cells, mast cells, macrophages, and CD4 T cells. Although found at lower levels in the B lymphocyte compartment, it is relatively enriched in germinal center and marginal zone B cells (16). In the central nervous system it is well expressed in microglial cells. There are two isoforms of murine RGS10 termed RGS10L (181 amino acids) and RGS10S (167 amino acids), although the former predominates in mouse leukocytes. While several RGS proteins use an amphipathic helix or cysteine string to target cellular membranes, RGS10 lacks these structures and relies on the palmitoylation of an n-terminal cysteine to target membranes (102). Loss of RGS10 in mice results in dysregulated microglial cell cytokine production with a pronounced increase in LPS induced pro-inflammatory cytokine production (103). Similarly, *Rgs10*$^{-/-}$ macrophages produce higher levels of pro-inflammatory cytokines in response to LPS treatment and exerted higher cytotoxicity towards neuroblastoma cells. The deficient macrophages exhibited a blunted M2 phenotype upon IL-4 priming (104). The role of RGS10 in lymphocytes has received relatively little attention. A gain of function/loss of function study using a human T cell line revealed that RGS10 inhibited chemokine receptor $G\alpha_i$-dependent T cell adhesion mediated by the integrins $\alpha_4\beta_1$ and $\alpha_L\beta_2$ (105). RGS10 regulated chemokine induced adhesion by limiting the activation of the Vav1-Rac1 pathway. RGS10 also limited T cell chemotaxis and inhibited chemokine induced activation of the small GTPase cdc42 (105). In depth studies of B and T cell function in RGS10 knock-out mice have not been reported.

3.3.5 RGS12—RGS12 is the largest protein of the RGS protein family and a member of the R12 subfamily along with RGS10 and RGS14 (see below). Full length RGS12 possesses a PDZ/PTB domain, post synaptic density disc-large zo-1 (PDZ) and phosphotyrosine binding (PTB) along with an RGS domain, 2 Ras binding domains (RBD), and a GoLoco motif. Alternative splicing creates multiple different isoforms of the RGS12 protein (106–108). In immune cell *Rgs12* mRNA expression is most prominent in dendritic cells with lesser amounts in macrophages (16). It is also strongly expressed in osteoclasts. In a recent study *Rgs12*$^{fl/fl}$ Mx1-Cre transgenic mice had the expression of *Rgs12* deleted at postnatal day 10 in interferon-responsive cells. This resulted in growth retardation, increased bone mass, and reduced numbers of osteoclasts in the mice (109). Similar results were found in *Rgs12*$^{fl/fl}$ *Cd11b-cre* mice, which would predominately delete *Rgs12* expression in neutrophils and macrophages (110). No assessment of dendritic cell function was performed in these mice. The N-terminal PDZ domain of RGS12 is known to interact with the C-terminus of CXCR2, however the functional importance of this interaction remains unknown (107). In muscle cells $G\beta\gamma$ signaling can lead to a PI 3-kinase-γ- and c-Src- dependent tyrosine phosphorylation of $G\alpha_i$. This results in the recruitment of RGS12 to $G\alpha_i$ and a reduction in its activity (111). Whether such a mechanism is operant in dendritic cells or macrophages has not been reported.

3.3.6. RGS13—RGS13 is a R4 RGS protein and it is one of the smallest of the RGS family (112,113). The human and mouse proteins are composed of 159 and 158 amino acids, respectively. Although largely composed on an RGS domain, RGS13 also interacts with and

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inhibits the activity of phosphatidylinositol-3-kinase (PI3K) in mast cells and phosphorylated cyclic AMP response element binding protein (pCREB) in B cells (114,115). RGS13 has one of the most restricted patterns of expression among the RGS proteins. In immune system cells *Rgs13* mRNA transcripts are largely confined to murine germinal center B cells and mast cells although they are also present in thymic medullary epithelial cells (16). Analysis of the B cell compartment in *Rgs13* deficient mice (C57BL/6 background) revealed that RGS13 constrains extra-follicular plasma cell generation, germinal center sizes, and germinal center B cell numbers (116). In an autoimmune strain of mice, BXD2, *Rgs13* is highly expressed in germinal center B cells, but also in T follicular helper (T_{FH}) cells (117). This contrasts with C57BL/6 mice where T_{FH} cells do not express detectable *Rgs13* mRNA. Surprisingly many of the B cell abnormalities noted in BXD2 mice were partially rescued by loss of *Rgs13* (117). While manipulating *Rgs13* expression in human B cell lines affected responses to chemokines, surprisingly, the loss of *Rgs13* expression did not materially affect murine germinal center B cell chemotaxis towards CXCL12 or CXCL13 (116). The reason for this is unclear although it may reflect the difficulties in assessing germinal center B cell chemokine responses. Stimulation of B cell adrenergic receptors results in the translocation of RGS13 to the nucleus, where it inhibits pCREB-mediated transcription (115). Germinal center B cells from *Rgs13* deficient mice expressed increased levels of several pCREB target genes (116,117).

3.3.7. RGS14—RGS14 is a member of the R12 subfamily of RGS proteins and a selective GAP for $G\alpha_{i/o}$. Mouse RGS14 is a 547 amino acid proteins that has a N-terminal RGS domain, two Ras binding domains, and a GoLoco motif. RGS14 can simultaneously bind GDP-$G\alpha_i$ via it GoLoco motif and act as a GAP for GTP bound $G\alpha_i$ (108,118–120). RGS14 can interacts with the monomeric G proteins Rap1, Rap2, and H-Ras (121). In the immune system *Rgs14* expression is abundant in neutrophils, but also found in most immune system cell types (16). In the brain *Rgs14* is expressed in neurons in the hippocampus and olfactory cortex, where it suppresses synaptic plasticity. Mice lacking *Rgs14* perform better than do wild-type mice in hippocampus-dependent tasks (91). The impact of the loss of *Rgs14* on the adaptive or innate immune system has not been reported.

3.3.8. RGS16—RGS16 is a member of the R4 family of RGS proteins. A cDNA that encodes RGS16 was initially cloned from the retina and subsequent studies demonstrated widespread tissue expression (82,122). It has a demonstrated role in the circadian regulation of intracellular G-protein signaling, which controls rhythmicity in the suprachiasmatic nucleus (123). Within cells of the immune system *Rgs16* mRNA is found at low levels in NK cells, CD4 T cells and platelets with higher levels in regulatory T cells (16). Several studies have suggested a role for RGS16 in allergic airway disease. T cell-specific *Rgs16* transgenic mice had reduced T cell trafficking to the lung following allergic airway while the lungs of allergen exposed *Rgs16* deficient mice had more TH2 cells than similarly challenged WT mice (124,125). *Rgs16* mRNA transcripts were strongly upregulated in human monocyte derived DCs treated with LPS and IL-10. Gain and loss of function experiments using a human monocyte cell line showed that RGS16 overexpression reduces the expression of pro-inflammatory cytokines while reduction of RGS16 had the opposite effect (126).

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3.3.9. RGS18—RGS18 is also an R4 RGS protein that is abundantly expressed in platelets and, to a lesser extent in megakaryocytes, macrophages, osteoclasts, neutrophils, and bone marrow progenitors (16,127–130). RGS18 inhibits both Gi- and Gq-mediated signaling. RGS18 has been implicated in the control of osteoclastogenesis mediated by RANKL by modulating signaling through the proton sensing GPCR OGR1 (131). Several recent studies have documented a role for RGS18 in thrombopoiesis and platelet function (132–136). Following exposure to thrombin or thromboxane A2 RGS18 is phosphorylated on serine 49 and serine 218, which promotes its binding to 14-3-3 proteins leading to an inhibition of RGS18 GAP activity. *Rgs18* deficient mice are mildly thrombocytopenic due to a decrease in platelet production, but are also hypercoagulable due to increased platelet sensitivity to platelet activators. These studies suggest that RGS18 modulates both megakaryocyte differentiation and platelet hemostatic function.

3.3.10. RGS19—RGS19 is a member of the RZ subfamily of RGS proteins (11,137). It is broadly expressed although at the highest levels in murine hematopoietic cells. Among immune cell types it is most prevalent in neutrophils, eosinophils, basophils, and mast cells. It is expressed at lower levels in lymphocytes, dendritic cells, natural killer cells, and macrophages (16). It has been implicated in cancer; described to suppress Wnt-dependent signaling and Ras-induced cell proliferation; and to be a downstream target of Notch signaling and involved in the activation of Akt (138–140). Like other RZ family members RGS19 has an amino-terminal cysteine string motif, an RGS domain, and a short carboxyl-terminal (141,142). Its GAP activity exhibits some specificity as it preferentially acts on $G\alpha_{i3}$ and $G\alpha_o$ showing little activity towards $G\alpha_{i2}$. It has also been shown to function as a GDI for $G\alpha_{i3}$ and $G\alpha_o$ even though it lacks a GoLoco motif (143). Thereby, RGS19 can retain GDP bound $G\alpha_{i3}$ following GTP hydrolysis. This would tend to suppress $G\alpha_{i3}$ activity, but potentiate $G\beta\gamma$ signaling. The results of targeting *Rgs19* in mice have not been reported, however knocking down *Rgs19* expression during mouse development suggested that Rgs19 functions to limit the expression of Wnt-responsive genes, which are needed for proper midline fusion of the mouse palate (144).

3.3.11 RGS insensitive $G\alpha_{i2}$—Because many cell types can express multiple RGS proteins the loss of an individual RGS protein may result in a minimal or no phenotype. Mapping the site of interaction of RGS proteins with $G\alpha_i$ proteins provided a partial solution to the problem of compensation by other family members (145). A single mutation in $G\alpha_i$ proteins was found to render them insensitive to RGS proteins as it blocked the interaction between the two proteins. This mutation does not affect $G\alpha_i$ binding to receptors, $G\beta\gamma$, or effectors; nor does it affect $G\alpha_i$ expression (146). Mice with such a mutation in the *Gnai2* locus have been made (147). This mutat ion results in a serine at posit ion 184 of the protein instead of the usual glycine ($G\alpha_{i2}$ G184S). The mutant $G\alpha_i$ protein is expressed at levels similar to that of the wild type protein. Study of these mice has revealed abnormal cardiac function and central nervous system dysfunction (148). Mirroring the *Rgs18* deficient mice, the mice carrying the $G\alpha_{i2}$ G184S mutation have enhanced platelet aggregation and increased platelet accumulation following vascular injury (149). Neutrophils with the $G\alpha_{i2}$ G184S mutation accumulated in the bone marrow and mobilized poorly to inflammatory sites. They displayed an enhanced sensitivity to background signals,

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altered kinetics of chemoattractant receptor signaling, and inappropriate CXCR2 downregulation. As neutrophils prominently express several RGS proteins, this phenotype has not been phenocopied by the loss of an individual RGS protein. The analyses of the G184S $G\alpha_{i2}$ neutrophils support a role for RGS proteins in setting a threshold for $G\alpha_{i2}$ activation, which helps to coordinate desensitization mechanisms (150). B cells from these same mice also had defective chemokine receptor signaling. The $G\alpha_{i2}$ G184S B cells had an elevated basal intracellular calcium level; enhanced non-specific migration, but poor chemokine (CXCL12, CXCL13, and CCL19) induced chemotaxis. Analysis of the intracellular calcium response and the activation of downstream effectors in the $G\alpha_{i2}$ G184S B cells indicated that both the onset of signaling and the termination of signaling were disrupted by the loss of $G\alpha_{i2}$/RGS protein interactions (151). As discussed earlier the loss of RGS proteins can affect both the activation and de-activation of GPCR signaling pathways (80). *In vivo* the $G\alpha_{i2}$ G184S B cells homed poorly to lymph nodes and exhibited improper *in situ* positioning, findings consistent with impaired chemokine receptor signaling. Thus, the RGS proteins in B cells act in a similar fashion to those in neutrophils helping to coordinate chemokine receptor $G\alpha_{i2}$ signaling and desensitization mechanisms (151). Surprisingly, the loss of $G\alpha_{i2}$/RGS protein interactions did not similarly affect signaling though another GPCR expressed in B cells, the sphingosine 1-phosphate receptor 1 (S1PR1) (151). The S1P/S1PR1 axis facilitates the egress of thymocytes from the thymus into the blood, and of B and T cells from lymph nodes into the efferent lymph, the latter a requirement for lymphocyte recirculation (152). In contrast to impaired chemokine receptor signaling, exposure of $G\alpha_{i2}$ G184S B cells to S1P elicited higher intracellular calcium responses and enhanced *in vitro* chemotaxis (151). Furthermore, there was no obvious defect in B cell egress from lymph nodes. This was perplexing as S1PR1 also signals by activating $G\alpha_{i}$. Perhaps explaining the discrepancy, S1PR1 preferred to pre-couple to $G\alpha_{i3}$ versus $G\alpha_{i2}$ (>10-fold difference), while the chemokine receptor CXCR4 had no such preference. This conclusion was based on an *in vitro* BRET (Bioluminescence Resonance Energy Transfer) assay using transfected proteins (151). Assuming that other B cell chemokine receptors also have no isoform bias, the higher expression of $G\alpha_{i2}$ versus $G\alpha_{i3}$ (5–10 fold difference) would lead to chemokine receptors being predominately pre-coupled to the mutant $G\alpha_{i2}$ protein, while S1PR1 would be pre-coupled more to the wild type $G\alpha_{i3}$, which would be subject to normal regulation by RGS proteins. Thus, signaling through S1PR1 via $G\alpha_{i3}$ would proceed normally, while the limited activation of $G\alpha_{i2}$ G184S might potentiate the signaling output. Further studies are needed to better understand the G-protein selectivity of the S1PR1 receptor and to better understand the role of RGS proteins in regulating $G\alpha_{i2}$ versus $G\alpha_{i3}$ signaling. Finally, macrophages carrying the $G\alpha_{i2}$ G184S mutation had a decrease in LPS-induced cytokine production when compared to wild type macrophages (153). This indicates that the loss of $G\alpha_{i2}$/RGS protein interactions in macrophages impacts TLR4 signaling and that RGS proteins dampen cytokine production. Whether this is a direct or an indirect effect of $G\alpha_{i2}$ on TLR4 signaling remains controversial.

4. Conclusions

Many of the GPCRs expressed by leukocytes function as chemoattractant receptors. The signals emanating from these receptors recruit leukocytes to inflammatory sites; organize the

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positioning of leukocytes in immune organs, help maintain the overall architecture of immune organs; coordinate the movements of leukocytes through tissues; and facilitate the trafficking of leukocytes into and out of lymph nodes, the bone marrow, skin, and the gut associated lymphoid tissues (GALT). The signaling output of GPCRs depends on the level of GPCR expression and the levels of heterotrimeric G proteins and their regulators. For chemoattractant receptors $G\alpha_i$ is crucial. Lymphocyte chemoattractant signaling is highly dependent upon $G\alpha_{i2}$, but absolutely dependent upon $G\alpha_{i2}$ and $G\alpha_{i3}$. In their absence murine B and T cells can no longer respond to these chemoattractant signals as other heterotrimeric G-proteins cannot substitute for their loss. Likely, $G\alpha_{i2/3}$ are similarly important in chemoattractant receptor signaling in other leukocytes, but this needs confirmation. This review focused on some of the regulatory mechanisms that impact $G\alpha_i$. Those that directly impact the receptor such as the G-protein regulatory kinases and β-arrestins were not covered, although they have considerable importance for chemoattractant receptor intracellular trafficking and receptor desensitization. The RGS proteins known to affect $G\alpha_i$ signaling in leukocytes were reviewed. The RGS domain-containing proteins appeared early in eukaryote evolution, they expanded in conjunction with the Gα subunits, and they have served as a primary mechanism to regulate the sensing of environmental signals by GPCRs. The considerable redundancy of RGS protein expression remains confounding although the analysis of the $G\alpha_{i2}$ G184S mice has provided important insights into the overall importance of RGS proteins expressed in neutrophils, platelets, and lymphocytes. Based on some of the studies reviewed here and others, RGS proteins have multiple roles in GPCR signaling. They set a threshold for $G\alpha_i$ activation, function to limit $G\alpha_i$ signaling, sharpen $G\alpha_i$ signaling output, coordinate with the β-arrestin system to control receptor desensitization, and is some instance serve as multi domain intracellular platforms for interactions with GDP-$G\alpha_i$, GTP-$G\alpha_i$, Gβγ, GPCRs, and other proteins.

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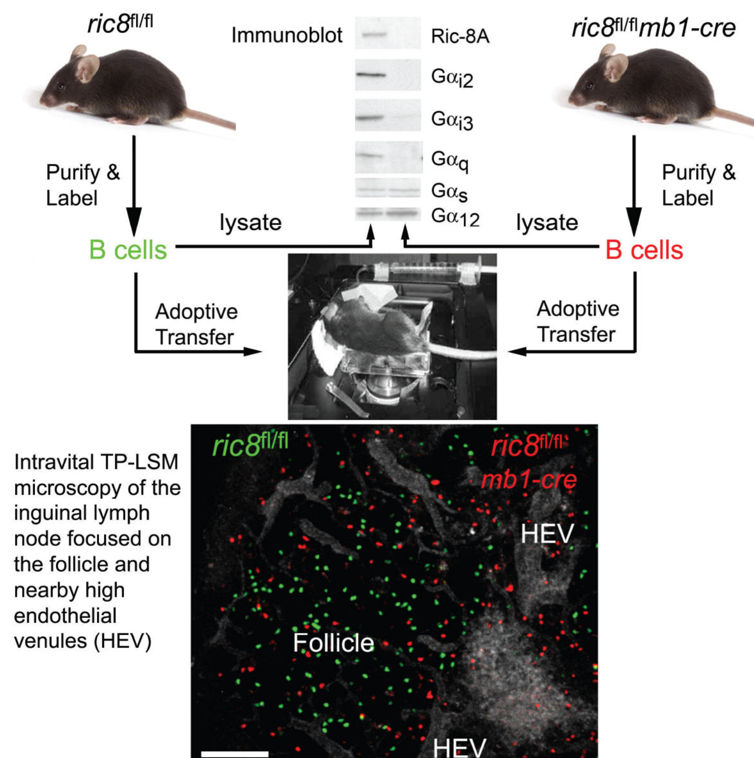

Fig. 1.

Intravital imaging of wild type and Ric-8A deficient B cells in the inguinal lymph node of a live mouse. Wild type (green) and Ric-8A deficient (red) B cells were adoptively transferred into a wild type mouse 24 hours before imaging. The mouse was anesthetized, a small incision made over the inguinal lymph node, and images were collected with a multiphoton microscope. The blood vessels including the high endothelial venules were outlined by injection of a fluorescent intravascular dye. A snapshot from the imaging is shown. Of not the Ric-8A deficient B cells localized near the HEVs and entered poorly into the lymph node follicle, a process that depends upon CXCR5 signaling. The expression of different Gα subunits in the wild type and Ric-8A deficient B cells is shown by immunoblotting cell lysates with specific antibodies.

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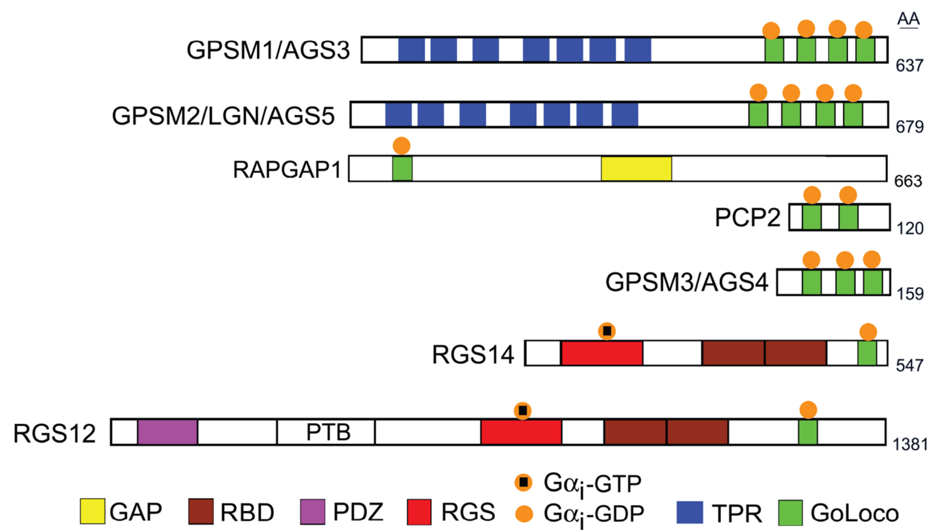

Fig. 2.

Schematics of the structures of GoLoco motif containing proteins. Proteins that contain GoLoco motifs (GPR domain) are shown. Abbreviations include RBD, Ras Binding Domain; PDZ, Post synaptic density Disc-large Zo-1; RGS, Regulator of G-protein Signaling; GAP, GTPase Activating Protein domain; and TPR, TetratricoPeptide Repeat. RGS domains bind GTP-Gα_i and accelerate its intrinsic GTPase activity while the GoLoco motifs bind GDP-Gα_i. The number of amino acids of the mouse versions of the indicated proteins is shown on the right.

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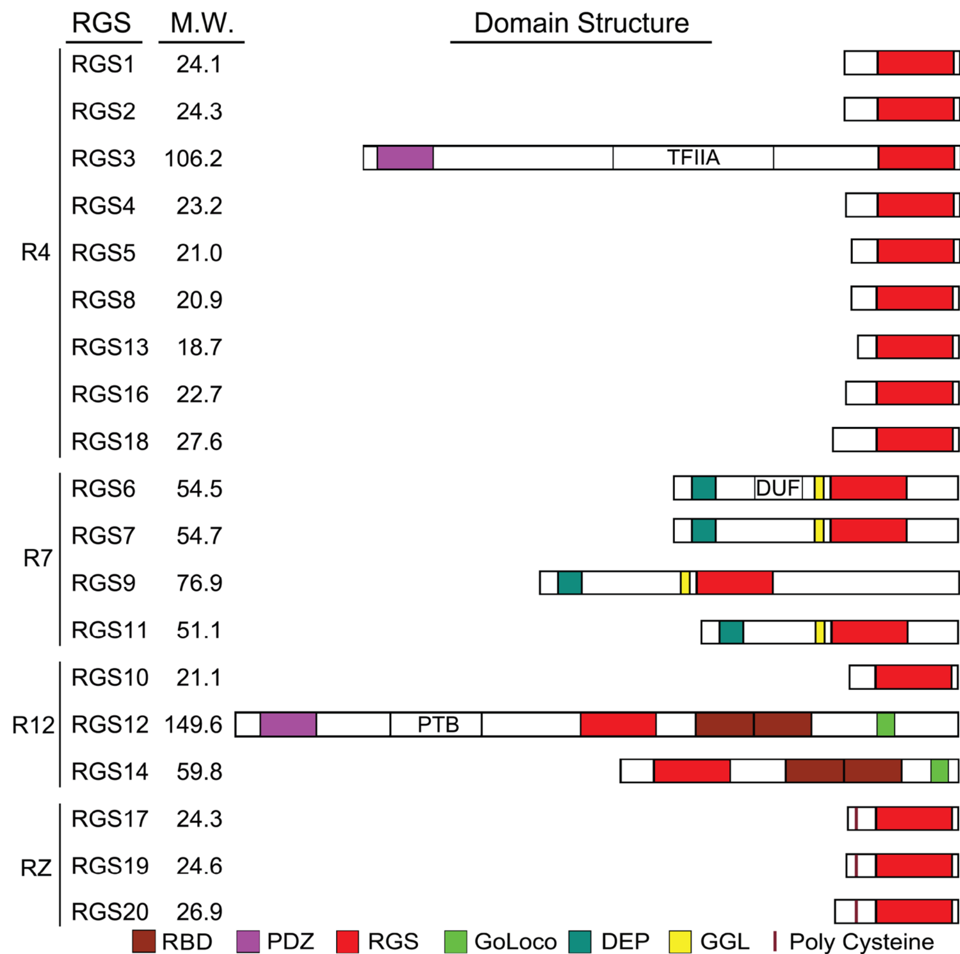

Fig. 3.

Schematics of the structures of the standard RGS domain containing proteins. Listed are the RGS domain containing proteins that act as GTPase activating proteins for $G\alpha_i$ and/or $G\alpha_q$. Abbreviations include RBD, Ras Binding Domain; PDZ, Post Synaptic density Disc-large Zo-1; RGS, Regulator of G-protein Signaling; PTB, PhosphoTyrosine Binding domain; DEP; Dishevelled, Egl-10 and Pleckstrin domain, GoLoco (GPR domain); GGL; G protein Gamma-Like domain, DUF; Domain of Unknown Function; and TFIIA, Transcription Factor IIA domain. Predicted molecular weights (M.W.) of the different proteins are shown.

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Table 1

Expression of different Gα subunits in various types of leukocytes[1]

Cell type	*Gnas*	*Gnai2*	*Gnai3*	*Gna12*	*Gna13*	*Gnaq*	*Gna11*	*Gna15*
B	12.1	13.7	2.3	0.3	3.0	0.4	0.4	0.1
Dendritic	8.2	11.8	2.3	0.5	2.4	1.4	0.3	0.1
Neutrophil	5.8	50.0	8.6	0.4	6.0	3.5	0.5	0.1
Macrophage	6.4	6.9	5.7	0.3	2.2	3.8	0.5	0.3
NK	16.1	13.7	3.3	0.3	2.0	0.2	0.5	0.2
NKT	6.1	3.5	2.5	0.1	3.2	0.2	0.4	0.1
CD4	7.7	11.8	2.7	0.2	2.8	0.1	0.4	0.1
CD8	8.9	7.2	2.7	0.2	3.0	0.1	0.5	0.1
Tγδ	11.0	6.9	3.6	0.3	4.1	0.2	0.3	0.1
Treg	6.0	7.1	3.2	0.3	3.3	0.3	0.4	0.1

[1] Relative mRNA expression in different cell types. Cells were isolated from 3 male C57BL/6J mouse spleens with the exception of the macrophages, which were isolated from the peritoneal cavity of similar mice. Data are from the Immunological genome project (http://www.immgen.org/databrowser/index.html) and based on RNA sequencing (Illumina) of mRNA from FACS purified cell samples. Very low or undetectable expression of *Gnat1*, *Gnat2*, *Gnat1*, *Gnai1*, *Gnao1*, *Gnaz*, *Gnal*, and *Gna14*. Cell type with highest expression of the different Gα subunits is highlighted. Levels of mRNA expression for Gβ and Gγ subunits can be found at the same website. For comparison β-actin expression is approximately 400. Values for each gene (x 10^3).

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Table 2

Expression of different RGS protein in various types of leukocytes[1]

Cell type	*Rgs1*	*Rgs2*	*Rgs3*	*Rgs10*	*Rgs12*	*Rgs14*	*Rgs16*	*Rgs18*	*Rgs19*
B	0.1	1.0	0.1	0.2	0.1	0.7	0.0	0.1	1.3
Dendritic	6.9	14.4	0.5	0.9	3.0	0.3	0.0	0.3	0.8
Neutrophil	0.1	28.2	1.0	0.1	0.0	3.4	0.0	0.7	3.1
Macrophage	0.1	3.7	0.3	2.2	0.0	0.3	0.0	1.2	1.0
NK	1.5	2.1	1.8	0.0	0.1	0.5	0.1	0.0	0.6
NKT	1.9	2.1	3.7	0.2	0.1	0.4	0.0	0.0	0.3
CD4	0.4	0.3	0.7	1.8	0.1	1.0	0.1	0.0	0.6
CD8	0.1	0.2	0.9	1.8	0.0	0.6	0.0	0.0	0.6
Tγδ	0.4	0.5	1.7	0.7	0.3	0.5	0.1	0.0	0.4
Treg	2.3	0.7	0.8	2.6	0.1	0.7	0.5	0.0	1.1

[1]Shown are the relative mRNA expression levels in different cell types. Cells were isolated from 3 male C57BL/6J mouse spleens with the exception of the macrophages, which were isolated from the peritoneal cavity of similar mice. Data are from Immunological genome project (http://www.immgen.org/databrowser/index.html) and based on RNA sequencing of mRNA from FACS purified cell samples. Cell type with highest expression of individual RGS protein mRNA expression is highlighted. Little or no expression for *Rgs4*, *Rgs5*, *Rgs6*, *Rgs7*, *Rrgs8*, *Rgs13*, *Rgs17*, *Rgs20*, or *Rgs22*. Non-detectible expression levels of *Rgs9* and *Rgs11* were found in the different cell subsets with the exception of CD4 and CD8 T cells, where low amounts were found (0.1). β-actin mRNA level is approximately 400. Values for each gene (x 10^3).

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Table 3

Impact of targeting $G\alpha_i$ regulatory genes expressed in murine leukocytes

Gene	Sites of expression[1]	Immune phenotypes[2]
Rgs1	DC, NK, NKT, Treg, Tγδ, GC B, Ba, MC	Altered antibody responses, B and gut T cell trafficking defects, colitis, enhanced macrophage accumulation in atherosclerotic plaques.
Rgs2	GN, DC, MF, NK, Ba, MC	Decreased platelet mediated hemostasis, decreased T cell proliferation, impaired antiviral immunity.
Rgs3	NKT, NK, GN, Tγδ, Treg	Defective T cell trafficking, altered cytokine production, enhanced T cell infiltration into the lung in an asthma model.
Rgs10	MF, Treg, T, DC, B, MC	Enhanced LPS induced inflammatory cytokine production by microglia, decreased M2 macrophage priming
Rgs12	DC, O	Increased bone mass with decreased osteoclast numbers.
Rgs13	GC B, MC	Increased extrafollicular antibody response, large germinal centers, decreased autoantibody production in autoimmune mice, increased mast cell chemotaxis.
Rgs14	GN, T, Treg, B	Normal T and B cell compartments[3]
Rgs16	Treg, T	Altered T cell trafficking and cytokine production in allergic lung inflammation.
Rgs19	MF, B, DC, T, Ba, MC	Altered antibody responses, B cell trafficking defects, expanded B cell compartment[4]
Gpsm1	Broadly in leukocytes, highest in NK	Reduced lymphocyte and dendritic cell chemotaxis. Impact on NK function unknown.
Gpsm2	GC B, double positive thymocytes	No reported phenotypes in leukocytes.
Gpsm3	Broadly in leukocytes, highest in GN	Myeloid cells had reduced migration to chemokines and enhanced apoptosis in vitro. No reported phenotypes in GN.
Ric8	Broadly in leukocytes, highest GN	B cell specific deletion resulted in a severe reduction in $G\alpha_i$, $G\alpha_q$, and $G\alpha_{13}$; and a severe B cell immunodeficiency.

[1]Based on mRNA expression, abbreviations as follows DC – dendritic cell, NK - natural killer cell, NKT – natural killer T cell, Treg- regulatory T cell, Tγδ-gamma delta T cell, GC B–germinal center B cell, MF- macrophage, GN – granulocyte, Ba – basophil, MC – mast cell, and O – osteoclast.

[2]Immune phenotypes for mice lacking the indicated genes. References are included in the text.

[3]Mice lacking Rgs14 have normal T and B cell compartments and normal antibodies responses (J. Kehrl, unpublished observation).

[4]Mice lacking Rgs19 expression exhibit altered antibody responses, B cell trafficking defects, and an expanded B cell compartment in the spleen (J. Kehrl, unpublished observation).