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Implications of Non-Canonical G-protein signaling for the Immune System

Cedric Boularan and John H. Kehrl*

B-cell Molecular Immunology Section, Laboratory of Immunoregulation, National Institutes of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD 20892

Abstract

Heterotrimeric guanine nucleotide-binding proteins (G proteins), which consist of three subunits α , β , and γ , function as molecular switches to control downstream effector molecules activated by G protein-coupled receptors (GPCRs). The GTP/GDP binding status of $G\alpha$ transmits information about the ligand binding state of the GPCR to intended signal transduction pathways. In immune cells heterotrimeric G proteins impact signal transduction pathways that directly, or indirectly, regulate cell migration, activation, survival, proliferation, and differentiation. The cells of the innate and adaptive immune system abundantly express chemoattractant receptors and lesser amounts of many other types of GPCRs. But heterotrimeric G-proteins not only function in classical GPCR signaling, but also in non-canonical signaling. In these pathways the guanine exchange factor (GEF) exerted by a GPCR in the canonical pathway is replaced or supplemented by another protein such as Ric-8A. In addition, other proteins such as AGS3-6 can compete with $G\beta\gamma$ for binding to GDP bound $G\alpha$. This competition can promote $G\beta\gamma$ signaling by freeing $G\beta\gamma$ from rapidly rebinding GDP bound $G\alpha$. The proteins that participate in these non-canonical signaling pathways will be briefly described and their role, or potential one, in cells of the immune system will be highlighted

1. Introduction

The positioning of immune cells in immune organs, the migration of cells that maintain immune surveillance, and the recruitment of innate and adaptive immune cells to sites of inflammation all depend upon signaling through chemokines receptors that couple to heterotrimeric G-proteins. In addition, many other mediators that affect immune cell functions also signal through G-protein coupled receptors (GPCRs). Some of these include histamine, purine nucleosides, C5A, prostaglandins, leukotrienes, serotonin, epinephrine, opioids, and certain phospholipids such as sphingosine-1-phosphate. The downstream effectors of heterotrimeric G-proteins, while limited in number, activate an array of signaling molecules, many shared with other signaling pathways known to affect immune

*To whom correspondence should be addressed: Dr. John H. Kehrl, Laboratory of Immunoregulation, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bldg. 10, Room 11B08, 10 Center Dr. MSC 1876, Bethesda, MD 20892; Fax: 301-402-0070. jkehrl@niaid.nih.gov.

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cell functions. Not surprisingly, GPCR/G-protein signaling is subject to finely developed regulatory systems that can target either the GPCR or the heterotrimeric G-protein. GPCRs, heterotrimeric G-proteins, and their regulators have been the subject of numerous reviews and several reviews have focused on GPCR/G-protein signaling in immune cell function [1, 2]. However, largely unknown to immunologists are the existence of non-canonical G-protein signaling pathways, which use other proteins to activate or modulate heterotrimeric G-protein subunit activity. These non-canonical signaling pathways have established roles in asymmetric cell division, mitotic spindle orientation, cytokinesis, and cell proliferation. Furthermore they intersect with GPCR-initiated signaling pathways. In this review, we provide a short introduction to heterotrimeric G proteins, outline how they are activated in the canonical model, and briefly, review some of their involvement in immune cell functions; but we will focus on GPCR independent G-protein signaling and its possible impact on cellular functions important for immune responses.

2. Basics about heterotrimeric G-proteins

Heterotrimeric G-proteins are GTPases composed of α -, β -, and γ -subunits. In humans, there are 21 $G\alpha$ subunits encoded by 16 genes, 6 $G\beta$ subunits encoded by 5 genes, and 12 $G\gamma$ subunits [3]. Heterotrimers are typically divided into four classes based on the primary sequence similarity of the $G\alpha$ subunit and the effector they activate: $G\alpha_s$ (stimulates adenylate cyclase), $G\alpha_i$ (inhibits adenylate cyclase), $G\alpha_q$ (activates phospholipase C β) and $G\alpha_{12/13}$ (triggers small Rho-GTPases). When an agonistic ligand binds a GPCR it induces a conformational change in the receptor that allows the receptor to mechanically function as a guanine nucleotide exchange factor (GEF). This promotes the release of GDP and the binding of GTP to the $G\alpha$ subunit. This exchange triggers the functional dissociation of the GTP bound $G\alpha$ subunit from the $G\beta\gamma$ dimer and from the receptor [4]. Both GTP- $G\alpha$ and $G\beta\gamma$ can then activate different signaling cascades (or second messenger pathways) and effector proteins. The $G\alpha$ subunit will eventually hydrolyze GTP to GDP by virtue of its inherent enzymatic activity, allowing it to functionally re-associate with $G\beta\gamma$ terminating signaling. A group of proteins termed Regulator of G-protein signaling (RGSs) act as GTPase-activating proteins (GAPs) for specific $G\alpha$ subunits. The RGS proteins accelerate the intrinsic GTPase activity of the $G\alpha$ subunit, thereby hastening the termination of the transduced signals [2]. In some instances the effector itself (i.e. phospholipase C β 1) also has GAP activity, which helps deactivate the signaling pathway [5].

Resolved crystal structures of $G\alpha$ proteins in various conformations have provided insights into the detailed mechanisms by which they cycle [6–8]. $G\alpha$ subunits have a conserved architecture composed of a GTPase domain and a helical domain. The GTPase domain is conserved among all the heterotrimeric $G\alpha$ proteins and is shared with monomeric G proteins. This domain hydrolyses GTP and provides the binding surfaces for the $G\beta\gamma$ dimer, GPCRs, and effector proteins. It contains three flexible loops named switch I, II and III, which undergo significant structural differences that depend upon the nucleotide binding status of $G\alpha$ [9–12]. The helical domain is unique to $G\alpha$ proteins and is composed of six α -helices that form a lid over the nucleotide-binding pocket burying the nucleotide in the core of the protein. All $G\alpha$ subunits with the exception of $G\alpha_i$ are N-terminally modified with the fatty acid palmitate. Members of the $G\alpha_i$ family are also myristoylated at the N terminus.

[13, 14]. This helps localize them to cell membranes. Their intracellular localizations have been discussed as they play important roles in GPCR independent $G\alpha$ activation [15]. The relative orientation of the $G\alpha$ and $G\beta\gamma$ subunits that leads to release of GDP from $G\alpha$ has been explained by two models. In the first model, the receptor mechanically uses the N-terminal helix of $G\alpha$ as a lever arm to pull $G\beta\gamma$ away from $G\alpha$. Thus, switches I and II from the $G\alpha$ subunit are pulled away from the nucleotide-binding pocket, which results in GDP release [16]. In support of this hypothesis, shortening of the αN helix by four amino acids at its C terminus to mimic the putative orientation induced by the receptor allows $G\beta\gamma$ to act as an exchange factor for $G\alpha_s$ [17]. The second, 'gear-shift' model proposes that the receptor uses the αN helix to force $G\beta\gamma$ into $G\alpha$, allowing the N terminus of $G\gamma$ to engage the helical domain of $G\alpha$. This interaction is postulated to cause an interdomain gap between the helical and GTPase domains of $G\alpha$ to open, which leads to GDP release [18].

3. Functions of heterotrimeric G-protein in immune cells (Figure 1)

3.1 $G\alpha_i$ in immune cells

The coordinated retention and relocation of leukocytes have key roles in the development, maintenance and proper functioning of the immune system and it is now well recognized that chemokines and their receptors are essential to it. Studies over the past 30 years using pertussis toxin (PTX), which irreversibly blocks $G\alpha_i$ mediated signaling, or more recently using gene targeted mice have demonstrated that $G\alpha_i$ has a non-redundant and essential role in leukocyte migration [19–24]. Targeted loss-of-function mutations of *Gnai1*, *Gnai2*, and *Gnai3* have been generated in mice revealing some redundancy as well as tissue specific functions for $G\alpha_{i1}$, $G\alpha_{i2}$, and $G\alpha_{i3}$ that partially depend upon the genetic background of the mice [25–29]. Cells of the adaptive and innate immune system express little $G\alpha_{i1}$ (based on data assembled from the immunological genome project [30], some of which is presented Tables 1–4), and no immune abnormalities have been reported in *Gnai1*^{−/−} mice. In contrast, the loss of *Gnai2* significantly impairs leukocyte trafficking and immune cell function. Loss of $G\alpha_{i2}$ augments the response of thymocytes to T-cell receptor (TCR)-mediated stimulation and results in an enhanced proliferation of double-positive (DP) thymocytes upon TCR ligation. This altered response promotes positive selection and likely rescue some thymocytes from death due to their inability to bind MHC molecules [31, 32]. *Gnai2*^{−/−} mice exhibit a significantly reduced fraction of DP thymocytes and an increased fraction of single-positive (SP) thymocytes. The absence of the $G\alpha_{i2}$ protein also causes a partial arrest of thymocyte differentiation at the double negative (DN) stage [33]. In addition, *Gnai2*^{−/−} mice have disordered B-cell subpopulations in the spleen with a reduction in marginal zone B cells and transitional T2 B cells, but an increase in follicular B cells. $G\alpha_{i2}$ is also important for the development and recruitment of B-1a and B-1b cells to the peritoneal cavity [34]. In mature T cells $G\alpha_{i2}$ signaling may negatively regulate T-cell proliferation [35–37]. Mature *Gnai2*^{−/−} T cells proliferated more and produced more inflammatory cytokines in response to stimulation with various mitogens [36, 38]. Interestingly, *Gnai2*^{−/−} T and B lymphocytes have decreased expression of the anti-apoptotic protein Bcl-2 and significantly increased levels of apoptosis; the size and number of Peyer's patches were also reduced [39, 40].

In contrast to the *Gnai2*^{-/-} mice, the *Gnai3*^{-/-} mice exhibit few if any defects in lymphocyte development or in their chemoattractant responses [21, 41]. However, the loss of *Gnai2* and *Gnai3* causes profound defects in B lymphocyte function and would likely severely impair the function of other leukocytes. *Gnai3*^{-/-} *Gnai2*^{flox/flox} mice crossed with *mb-1 cre* mice resulted in mice that lacked Gα_{i2} and Gα_{i3} in their B lymphocytes. Their absence profoundly disturbed the architecture of lymphoid organs with a loss of B cell compartments in the spleen, thymus, lymph nodes, and gastrointestinal tract. It also resulted in a severe disruption of B cell function and a hyper-IgM like syndrome. The deficient B cells were refractory to chemokine stimulation and poorly responsive to antigen receptor engagement [41]. While the major phenotypes in mice lacking Gα_i in lymphocytes result from impaired signaling through chemokine receptors, it is likely that impaired signaling from other Gi-linked receptors and the absence of non-canonical Gα_i-linked signaling pathways contribute to the defects.

3.2 Gα_q in immune cells

The initial study of *Gnaq*^{-/-} mice found that immune cell subsets and numbers were not significantly different from those observed in wild-type (WT) animals and no evidence of immune system abnormalities were found in the mice [42]. However, a more detailed analysis of B cells in these mice revealed that Gα_q regulates the transition between bone marrow pro- and pre-B cells and affects the development of follicular and marginal zone B cells in the spleen. The transitional 1 (T1) B cells and marginal zone B (MZB) cells of Gα_q-deficient mice were found to be largely resistant to anti-IgM-induced cell death [43–45]. *Gnaq*^{-/-} B cells were also less susceptible to B-cell-activating factor (BAFF) withdrawal. Likely as a consequence, *Gnaq*^{-/-} B cells out-competed wild type B cells in bone marrow mixed chimeras [44]. Moreover, low levels of Gα_q expression in peripheral blood lymphocytes from rheumatoid arthritis patients correlated with Mcl-1 upregulation, decreased caspase-3 activity, and decreased apoptosis [46]. The specific Gq-linked receptors or pathways that affect the sensitivity of lymphocytes to apoptotic signals remain obscure. There is also evidence that some chemokine receptors can signal through Gq. The recruitment of chemokine receptors to the immune synapse resulted in their re-coupling to Gq, which enhanced T cell proliferation and cytokine production [47].

3.3 Gα_{12/13} in immune cells

The expression of the RGS domain of p115RhoGEF, which inhibits signaling through G_{12/13} coupled receptors, and the expression of Gα₁₃ and Gα₁₂ minigenes, which specifically inhibit signaling through G₁₂ and G₁₃ linked receptors, in thymocyte cultures revealed a role for Gα₁₃ in thymocyte development. Inhibition of G₁₃ signaling caused a block at the DN3 stage of thymocyte development with reduced thymocyte survival and proliferation [48]. Mice with the specific inactivation of Gα₁₂ and Gα₁₃ in B lymphocytes had a reduction in splenic MZB cells and a delayed antibody response to thymus-independent antigens. Furthermore, migration in response to sphingosine 1-phosphate was strongly increased in the MZB cells, but not in follicular B cells from the mutant mice [49]. T cell-specific deletion of Gα₁₂ and Gα₁₃ caused lymphadenopathy and increased T cell entry into lymph nodes; enhanced T cell proliferation; and an increased susceptibility toward T cell-mediated diseases. The T cell specific deletion of Gα₁₂ and Gα₁₃ enhanced the

activity of the Rac1 and Rap1, but reduced that of RhoA [50]. While the loss of G $\alpha_{12/13}$ in lymphocytes causes significant phenotypes in both the B and T lymphocyte compartments the specific receptors responsible for these phenotypes are largely unknown.

3.4 G α_s in immune cells

The stimulatory G α subunit (G α_s) of the heterotrimeric G protein Gs plays a central role in GPCR-mediated signal transduction by coupling receptors to the activation of adenylyl cyclase (AC) and increased synthesis of cAMP. Increases in cAMP can inhibit T cell function, i.e. blunt CD4⁺ T cell activation, proliferation, and the production of certain cytokines such as IFN- γ and TNF- α [51]. In contrast to these data, cAMP has also been shown to stimulate inflammation by promoting Th17 cell expansion [52]. For example, the mucosal adjuvant cholera toxin (CT) acts via a cAMP-dependent mechanism to provoke Th17 immune response at mucosal sites [53]. Helping to resolve such discrepant findings, CD4 T cell specific deletion of G α_s led to CD4 T cells that had reduced cAMP levels, decreased Ca²⁺ influx, and weak Th17 and Th1 responses, but normal Th2 and Treg responses both *in vitro* and *in vivo* [54]. Again the Gs-coupled receptors responsible for these phenotypes are unknown.

4. Receptor Tyrosine kinase as activators of heterotrimeric G-protein

Non-canonical activation of G α proteins by receptor tyrosine kinases (RTKs) may be defined as either a direct activation process involving an interaction of the G α protein with a RTK or functional cross-talk in the form of transactivation through the RTK [55]. A detailed review of those receptors is available [56], here we will only mention the RTK expressed in immune cells based on data assembled from the immunological genome project [30] and presented in Tables 1–4.

4.1 EGFR (epidermal growth factor receptor) and FGFR (fibroblast growth factor receptor)

Among immune cells EGFR and FGFR mRNA expression is primarily detected in dendritic cells and macrophages (Tables 1–4). The EGFR is known to be required for human cytomegalovirus entry into monocytes and EGFR signaling promotes cellular motility [57] while in macrophages FGFR enhances tumor cell migration and invasion in a CXCR2-dependent manner [58]. G α_{i1} and G α_{i3} are needed for EGF-mediated activation of the AKT-mammalian target of rapamycin complex 1 (mTORC1) pathway as *Gnai1*^{-/-} and *Gnai3*^{-/-} mouse embryo fibroblasts (MEFs) responded very poorly to EGF stimulation. Treatment of wild type MEFs with pertussis toxin failed to inhibit the EGF-induced AKT activation arguing that G α_i nucleotide exchange was unnecessary or occurred via a non-GPCR GEF. G α_{i1} and G α_{i3} were shown to form a complex with Grb2-associated binding protein 1 (Gab1), which was required for EGF-induced Gab1 phosphorylation and its subsequent interaction with the regulatory (p85) subunit of phosphatidylinositol 3-kinase (PI3K). As a result, EGF-induced cell migration and proliferation were compromised in the absence of G α_{i1} and G α_{i3} [59]. These results are consistent with previous observations that showed that macrophages production of cytokines and chemokines were adversely affected by the loss of G α_{i3} . [60]. The role for FGFR in macrophages remain to be determined as the crosstalk between G α_s and FGFR has only been described in adipocytes [61]

4.2 IGFR1 (insulin growth factor receptor), IGFR2 and InsR1 (insulin receptor)

IGF1R, IGF2R, and insulin receptors are expressed at varying levels in the different cell subsets we analyzed (Tables 1–4). The role of IGF1R signaling in lymphocytes was assessed by analyzing RAG2-deficient mice reconstituted with *Igf1r*^{-/-} fetal liver cells. Despite the absence of IGF1R, the development and *ex vivo* activation of B and T lymphocytes were unaltered in these mice, but the humoral immune response to the T cell-independent antigens was reduced, while the responses to the T cell-dependent antigens were normal [62]. Another study found that the activation of the MAP kinase pathway by IGF1R was inhibited by PTX treatment or by an inhibitor of Gβγ-mediated signaling [63]. Although not shown in immune cells, IGF2R was found to mediate signals in a PTX-sensitive and PKC-dependent manner [64]. While no direct binding of Gα_{i/o} proteins to the IGF2R was reported in this study [65], the authors found that a portion of the IGF2R cytoplasmic domain could interact with Gα_{i2}. Furthermore, the insulin receptor was found to directly bind Gα_i proteins [66] and insulin-induced activation of NADPH-dependent H₂O₂ generation in human adipocyte plasma membranes was mediated by Gα_{i2} [67]. While the majority of these studies were carried out in cell types not associated with innate or adaptive immune function, they raise the possibility that the non-receptor tyrosine kinases commonly found in immune cells may alter the activity of Gα subunits.

4.3 CSFR (colony stimulating growth factor receptor)

CSFR mRNA expression is abundantly detected in monocytes and macrophages, where it functions as a receptor for M-CSF, a secreted cytokine that influences hematopoietic stem cell differentiation to macrophages and related cell types. The evidence that alteration of heterotrimeric G protein function affects signal transduction of hematopoietic cytokines comes from experiments done using BAC 1.2F5 cells, a murine macrophage cell line that is dependent from monocyte-macrophage colony-stimulating factor (CSF1) for its proliferation. Constitutively activate Gα_{i2} (Gα_{i2}-Q205L) reduced the cells requirement for CSF1 and protected the cells from death by apoptosis induced by CSF1 withdrawal whereas, expression of Gα_{i2}-G204A, a dominant negative mutant, inhibited BAC 1.2F5 cell proliferation in response to CSF1 [68]. Whether Gα_i proteins have a role in the expansion of normal hematopoietic progenitors remains to be determined.

5. Exogenous GPCR independent activation of heterotrimeric G-protein

5.1 Aluminum Fluoride regulates TCR and BCR signaling

In the 1990's a series of publications provided support for the involvement of G proteins in lymphocyte antigen receptor signaling (BCR and TCR) by treating cells with aluminum fluoride (AlF₄⁻). Two studies examined the effect of aluminum fluoride on T cells and suggested that TCR signaling might engage heterotrimeric G-proteins [69, 70]. In addition, studies of BCR signaling revealed enhanced tyrosine phosphorylation of Igα and Igβ, components of the BCR, following aluminum fluoride treatment [71]. These studies relied on the known effect of AlF₄⁻ on heterotrimeric G-proteins [72]. By binding GDP bound Gα near the site occupied by the γ-phosphate, the AlF₄⁻-GDP-Gα complex mimics GTP-Gα [73, 74]. However, the action of AlF₄⁻ is not limited to GDP-bound Gα subunits, but other small G proteins can also be activated. Furthermore, other enzymes bound to GDP or ADP

can potentially complex with AlF_4^- imitating ATP or GTP bound enzymes. For example, a mitochondrial ATPase can be targeted by AlF_4^- [75] as well GDP bound tubulin [76]. In retrospect, these studies of the effect of AlF_4^- on TCR and BCR signaling provide little support for a direct role for $\text{G}\alpha$ proteins in antigen receptor signaling, but more likely implicated small GTP binding proteins.

5.2 Pasteurella multocida toxin

Pasteurella multocida toxin (PMT) is produced by toxigenic strains of the Gram-negative *Pasteurella multocida* bacteria. The toxin specifically targets the α -subunits of heterotrimeric G proteins. These bacteria can be isolated from chronic respiratory infections in animals and the toxin is the likely cause of atrophic rhinitis in pigs. The bacteria can infect humans, but usually following the bite of an infected animal [77]. PMT is known to activate $\text{G}\alpha_q$, $\text{G}\alpha_i$ and $\text{G}\alpha_{13}$ by deamidating a glutamine in the switch II region [78–80]. This causes the constitutive activation of the α -subunit and release of the $\beta\gamma$ -subunit from the heterotrimeric G protein. While initially activating G-protein mediated signaling pathways, prolonged exposure of cells to PMT causes G-protein degradation and impaired G-protein signaling [81]. The acute treatment of Swiss 3T3 cells with PMT caused the mobilization of intracellular calcium, protein kinase C (PKC) activation, and enhanced cell proliferation [82]. PMT treatment also triggered the activation of the JAK/STAT signaling pathway via $\text{G}\alpha_q$ activation [83]. Because of the importance of the JAK/STAT pathway in immune cells several studies have examined the effect of PMT on immune cells. PMT was shown to promote the maturation of human monocyte-derived dendritic cells through the activation of phospholipase C [84]. PMT treatment suppressed IL-12p40 production by LPS activated monocytes and impaired their ability to stimulate T cells. This inhibition was mediated by augmented $\text{G}\alpha_i$ - and $\text{G}\beta\gamma$ -signaling [85]. While *in vivo* injection of PMT in mice did not affect the antibody response to OVA, it did suppress the adjuvant activity that Cholera toxin had [84]. The treatment of dendritic cells with PMT resulted in the activation of small GTPases, promoted actin polymerization, and inhibited cell migration [86]. Despite these results further studies of the effects of PMT on lymphocytes and other innate immune cell types is warranted.

5.3 Mastoparan and activated-GPCR mimicking peptides

Mastoparan is a peptide toxin from wasp venom whose amino acid sequence is Ile-Asn-Leu-Lys-Ala-Leu-Ala-Ala-Leu-Ala-Lys-Lys-Ile-Leu. [87]. Mastoparan promotes mast cells histamine secretion and platelet serotonin release. It does so by promoting the dissociation of GDP from $\text{G}\alpha$ proteins, which promotes GTP binding. As a consequence, $\text{G}\alpha$ GTP turnover is increased independent of any GPCR engagement. In the setting of a phospholipid membrane the toxin adopts a structure that resembles an activated GPCR [88]. Substance P is an 11 amino acid peptide that was initially thought to use a similar strategy to activate G-proteins [89], however, a GPCR was identified that mediates many of its effects. Substance P can potentiate human T lymphocyte proliferation [90], enhance the rat regional lymph node antibody response [91], promote phagocytosis [92], and increase cytokine production by human monocytes [93]. Using a phage display strategy a series of GDP-selective $\text{G}\alpha$ binding peptide have been discovered that trigger $\text{G}\alpha_i$ nucleotide exchange. Structural analysis of a $\text{G}\alpha_{i1}$ /peptide complex revealed unique changes in the $\text{G}\alpha_i$ switch regions that

were predicted to enhance nucleotide exchange by creating a GDP dissociation route [94]. Whether such peptides can be successfully delivered to cells to modulate G-protein signaling remains to be determined.

5.4 GTP γ S

GTP γ S (guanosine 5'-[γ -thio]triphosphate) is a non-hydrolyzable analog of GTP. The substitution of sulfur for one of the oxygens of the γ -phosphate of GTP creates a nucleotide that is poorly hydrolyzed. This prevents the GTP binding proteins from being deactivated. When introduced into patch-clamped mast cells GTP γ S stimulated exocytosis independently of phospholipase C (PLC) [95]. Although the activity of GTP γ S is not restricted to heterotrimeric G protein, PTX inhibited the GTP γ S induced exocytosis. This implied the involvement of a PTX-sensitive heterotrimeric G protein [96]. Another study implicated G α_{i3} in mast cell exocytosis as the introduction into cells of a synthetic peptide that corresponded to the carboxyl-terminus of G α_{i3} specifically blocked secretion [97]

6. Endogenous GPCR-independent activators of heterotrimeric G-protein

Activators of G-protein signaling (AGS) proteins were initially identified in a functional screen for cDNAs that activated G-protein signaling in the absence of a seven-membrane span receptor in *Saccharomyces cerevisiae* [98, 99]. AGS proteins were mechanistically divided into three groups: GEFs (group I, a solitary member AGS1), guanine nucleotide dissociation inhibitors, GDIs (group II, four members AGS3–6); and proteins that interact with G $\beta\gamma$ (group III, 5 members AGS2 & AGS7–10) [100]. Many of these AGS proteins are well expressed in immune cells (Tables 1–4). Over the years the list of proteins that can promote the dissociation of GDP from G α has been growing (Group 1 proteins). This includes the resistance to inhibitors of cholinesterase 8 (Ric-8) proteins (Ric-8A, Ric-8B, Ric-8B 9), G α -interacting vesicle-associated protein (GIV, also called Girdin or CCDC88a), GAP-43/neuromodulin/B-50, cysteine string protein (CSP), human phosphatidylethanolamine-binding protein (hPEBP), β -amyloid precursor protein, presenilin-1 and tubulin. In addition, nucleobindin 1 and nucleobindin 2, which contain regions homologous to a motif in GIV responsible for G α binding and GEF activity, have been shown to produce a measurable increase in steady-state G α_{i3} GTPase activity. Several of these proteins will be described and their role or their potential function for the immune system will be highlighted.

6.1 AGS3, GIV, and autophagy

Macroautophagy or autophagy is a general and evolutionary conserved response to starvation and stress activated in eukaryotic cells [101]. Furthermore, it serves critical functions in both innate and adaptive immunity. In macrophages, autophagy can be triggered by the engagement of pattern recognition receptors. It helps to eliminate many intracellular pathogens [102] and impacts inflammation by regulatory interactions with innate immune signaling pathways [103]. It contributes to antigen presentation by antigen presenting cells [104], affects T cell homeostasis, shapes the T cell repertoire [105], and modulates T cell subset function [106]. In humoral immunity, autophagy is needed for the survival of B1

cells, a subset of self-renewing B cells that cannot be replenished from the adult bone marrow [107].

A series of studies implicated AGS3, $G\alpha_{i3}$, GIV, and an RGS protein, RGS19, in the regulation of autophagy in human cell lines. In the colon cancer cell line HT-29 the rate of autophagic flux was low when $G\alpha_{i3}$ was GTP bound and stimulated when GDP bound [108–110]. RGS19 (GAIP), which enhances the hydrolysis of GTP by $G\alpha_{i3}$ proteins, increased the rate of autophagy [111], while AGS3 controlled an early step in the autophagic pathway prior to the formation of the autophagosome [112]. The protein GIV acted as a GEF for $G\alpha_{i3}$ [113] and it inhibited autophagy by preventing the binding of $G\alpha_{i3}$ to AGS3 [114]. Moreover, GIV was essential for the chemotaxis of HeLa cells and the human macrophage cell line THP-1. It promoted AKT activation and the remodeling of the actin cytoskeleton [115]. A recent study evaluated the role of AGS3, RGS19, and $G\alpha_{i3}$ in the regulation of autophagy in bone marrow derived macrophages using cells prepared from gene targeted mice. While these proteins are well expressed in wild type macrophages, the levels of basal autophagy, autophagic induction, autophagic flux, autophagic degradation, and the anti-autophagic action in macrophages were unaltered in macrophages that lacked either $G\alpha_{i3}$, AGS3, or RGS19 [116]. Thus, although these proteins affect autophagy in some cell types, they do not in murine macrophages. Further studies will be needed to determine whether these proteins regulate autophagy in other cells that participate in innate and adaptive immunity.

6.2 AGS1

AGS1 also known as RASD1 or DEXRAS1 is with RASD2 (Rhes) a member of the Ras superfamily of small G-protein. AGS1 increases GTP γ S binding to purified $G\alpha_i$ and $G\alpha_o$ [117], but AGS1 expression in immune cells is very low making it an unlikely candidate to tune the $G\alpha$ activity (Table 1 & 2).

6.3 Tubulin

Tubulin differs in its mechanism of G protein regulation as it increases GTP binding to $G\alpha$ subunits by the exchange of GTP bound to tubulin for the GDP bound to $G\alpha$ [118, 119]. Tubulin also interacts with $G\beta\gamma$ subunits [120]. Although not fully delineated, the tubulin interaction with receptors and G protein subunits may play multiple roles in G-protein trafficking, G-protein activation, and the control of microtubule dynamics at the contact area where cytotoxic T lymphocytes interact with their target cells [121]. The role of heterotrimeric and non-canonical G protein signaling proteins might be an interesting area of investigation during cytotoxic T lymphocytes activity. Ric-8 and $G\alpha_i$ are known to coordinate the recruitment of AGS5 (LGN) and dynein to the cell cortex to help orient the mitotic spindle [122] and dynein helps position the centrosome during immunological synapse formation in T cells [123].

6.4 Ric-8A and Ric-8B

The Ric-8 protein was first identified in *Caenorhabditis elegans* as its deficiency allowed the organism to survive treatment with cholinesterase inhibitors [124]. Single isoforms of Ric-8 have been found in *Caenorhabditis elegans* and *Drosophila melanogaster*, whereas

two Ric-8 genes have been found in mammals: Ric-8A and Ric-8B. A recent review provides a good summary of the discovery of Ric-8 proteins and their roles in model organisms [125]. Ric-8A acts as a GEF for free $G\alpha_{i/o}$, $G\alpha_q$, and $G\alpha_{12}$ proteins with no apparent effect on $G\alpha_s$ nucleotide exchange [126]. Full length Ric-8B is primarily a GEF for $G\alpha_s$ and its homologue $G\alpha_{olf}$ although *in vitro* Ric-8B also can have an appreciable effect on $G\alpha_q$ and $G\alpha_{13}$, but not on $G\alpha_{i1}$ [127]. The shorter splice variant Ric-8B₉ appears to be a significant GEF for $G\alpha_s$ only [127, 128]. Ric-8 proteins bind preferentially to their $G\alpha$ targets in the GDP-bound state, and promote GDP dissociation to form a stable nucleotide-free transition state complex. Ric-8 proteins do not act on $G\alpha$ bound to $G\beta\gamma$ (i.e. heterotrimeric G-proteins), but can trigger nucleotide exchange on free $G\alpha$ subunits or when bound to an AGS protein such as Ags3, Ags4, Ags5, or RGS14 [126–130].

The roles of Ric-8 proteins in hematopoietic cells and in the adaptive and innate immune systems are unknown. One study has suggested a role for Ric-8B in osteoblasts, but no other relevant studies have been published. Based on its known role in asymmetric cell division in developing model organisms, Ric-8 proteins may be functionally important in hematopoietic cell development and differentiation. Ric-8 proteins may also affect GPCR signaling in immune cells. There is evidence that GPCR linked signaling pathways can be impacted by Ric-8 and AGS proteins, see below. Finally, the chaperone activities of Ric8A and Ric-8B for $G\alpha$ subunits are likely necessary for the delivery of $G\alpha$ subunits to proper intracellular sites in lymphocytes and other cell types that participate in adaptive and innate immunity. Each of these areas is discussed below.

6.4.1 Osteoimmunology and Ric-8B—In the bone marrow HSC (hematopoietic stem cells) differentiation occurs in close proximity to endosteal osteoblasts [131]. The Ric-8B gene is highly expressed in proliferating pre-osteoblastic cells and negatively regulated by an isoform of the transcription factor C/EBP β during osteoblast differentiation. Transient overexpression of Brg1 or Brm, the catalytic subunits of the SWI/SNF chromatin-remodeling complex, inhibited Ric-8B promoter activity [132]. However, the role of the abundantly expressed Ric-8B in the pre-osteoblastic cells and functional importance of the downregulation during differentiation is unknown. Based on the known role of Ric-8B as a $G\alpha_s$ chaperone, the decline in Ric-8B is likely to be accompanied by a reduction in $G\alpha_s$ levels. An inspection of Ric-8B mRNA levels in different hematopoietic stem cells and progenitors did not reveal much variation (Table 1–4).

6.42 Asymmetric cell division—Symmetric or asymmetric cell divisions refer to the mode of cell division that results in two phenotypically identical- or different-daughter cells, respectively. The phenotypic distinguishing features may be the cell size, cell surface receptors, intracellular proteins, transcription factors, or messenger RNAs (for extensive review [133]). Hence, these phenotypic differences provide the basis for the functional differences in the daughter cells, i.e., their cell fates. Following encounter with their antigen, naïve T lymphocytes go through well-orchestrated series of divisions generating different populations of cells that fulfill immediate effector functions or generate long-lived immunological memory. Two basic models exist to explain the generation of such functionally distinct T cell phenotypes: the “one naïve cell – one fate” model and the

asymmetric model. In the asymmetric model asymmetric cell division has been proposed as the mechanism that allows naïve T cells to give rise to two different daughter cells. These are referred to as proximal or distal daughter cell depending on their proximity to the APC (antigen presenting cell). Such asymmetric T cell division represents a process that allows single cells to give rise to two, phenotypically and functionally different daughter cells, and thereby permits diversification of cell populations. Actually these 2 models are not mutually antagonistic. Different circumstances and/or environments may favor one version versus the other. Asymmetric distribution of proteins has been documented both during T cell stimulation and division [134–136] and following B cell activation [137]. The alignment of the mitotic spindle with the axis of cell polarity generally requires AGS5 (LGN, GPSM2) [138]. As indicated above a series of publications have highlighted the role of Ric-8 during asymmetric cell division in *Caenorhabditis elegans* and *Drosophila melanogaster* [139–142]. Together with its role in the recruitment of AGS5/LGN and dynein to the cell cortex to help orient the mitotic spindle [122], Ric-8A is a candidate to help tune T and B cells asymmetric fates after their activation. Of note AGS5/LGN is highly enriched in double positive thymocytes and in germinal center B cells (Table 1). The thymus and germinal center are sites where B and T cells proliferate and are selected for different cell fates, respectively.

6.43 Ric-8 in cell signaling—Considerable evidence in other cell types supports a role for both Ric-8A and Ric-8B in GPCR-dependent signaling. For example, Ric-8B was found to boost cAMP production triggered by odorant receptors, which couple to Golf [128]. Ric-8A amplified a Gq-linked GPCR mediated activation of ERK [143]. Silencing of Ric-8A in embryonic fibroblasts inhibited PDGF-induced cell migration and prevented the translocation of $G\alpha_{13}$ to the cell cortex [144]. Finally, two studies have implicated AGS proteins, AGS3 and AGS4, in chemotaxis [145, 146]. Although neither study examined the role of Ric-8A, a nonreceptor GEF is likely needed to trigger the release of $G\alpha_i$ from the AGS protein. Because of the importance of chemotaxis and cell migration for immune cell trafficking and function, an important function for Ric-8A may be to help tune cell responsiveness to chemoattractants.

6.44 G α protein synthesis and stabilization—As described earlier physical loss of G α proteins have drastic effects on the host immune system. Recent studies have proven that Ric-8 proteins can facilitate G α expression and localization at the plasma membrane. Early observations in *Drosophila melanogaster* [147] and in *Caenorhabditis elegans* [139] suggested that Ric-8 served as a chaperone, which promoted heterotrimeric G-protein mRNA translation and protein stabilization. In addition, Ric-8B boosted the expression of non-tagged olfactory receptors in heterologous cell systems [148] and it stabilized $G\alpha_s$ subunits by preventing their ubiquitination and subsequent proteasome-mediated degradation [149]. In another study, Ric-8A stabilized nascent $G\alpha_i$ proteins at the level of the endoplasmic reticulum [150]. These data led to the use of Ric-8 co-expression as a means to improve the purification of G α from cell systems [151]. The molecular chaperone activity of Ric-8A and Ric-8B has also been assessed using Ric-8A^{-/-} and Ric-8B^{-/-} embryonic stem cell lines as the disruption of the coding sequences in mice caused early embryonic lethality [152]. Heterotrimeric G-proteins localized to the plasma membrane in

the embryonic stem cells lacking Ric-8, but at markedly reduced levels compared to their levels in wild-type cells. Short $G\alpha_i$, $G\alpha_q$, and $G\beta_1$ half-lives in the deficient cells contributed to the reduced levels of the heterotrimeric G-proteins. These data indicate that Ric-8A and Ric-8B help protect $G\alpha$ subunits from degradation, facilitate their proper folding, and contribute to their membrane targeting [153, 154]. The reduction or loss of Ric8 proteins in immune cells would likely have dire consequences for those cells due to the concomitant loss of $G\alpha$ proteins.

6.5 Nucleobondin 1 and 2 (Calnuc and NucB2)

Calnuc (nucleobindin 1 or NUCB1) was identified as a $G\alpha$ -binding protein in a yeast two-hybrid screen using $G\alpha_{i2}$ as bait [155]. Calnuc and NUCB2, a highly homologous calcium-binding protein, share a common motif with GIV for $G\alpha_i$ binding and activation [156]. Their role in immune cells is unknown, but by impacting calcium signaling these proteins may help fine-tune calcium dependent events following the engagement of antigen receptors, during phagocytosis, or inflammasome activation. The coding region for NUCB1 is well expressed in murine macrophages and lower levels in lymphocytes, while mRNA that encodes NUCB2 is found at high levels in plasmacytoid dendritic cells, and at lower levels in marginal zone B cells and neutrophils (Tables 1–4).

6.6 Presenilin-1 (Psen-1)

Mutations in presenilin 1, a transmembrane protein, have been linked to early onset of Alzheimer disease. This may be secondary to Psen-1 dependent gamma-secretase activity for amyloid- β protein (β -APP) processing [157, 158]. As a component of gamma-secretase activity, Psen-1 is also involved in signaling by the transmembrane Notch proteins, which function in T and B cell homeostasis and lymphocyte development [159]. Biochemical analyses of a gain of function Psen-1 knock-in mice found a higher level of spontaneous lymphocyte apoptosis in the mutant mice compared to wild-type mice. In addition, lymphocytes from the Psen-1 mutant mice exhibit a spontaneous elevation of cytosolic Ca^{2+} concentration, and increased vulnerability to apoptotic signals [160]. β -APP and Psen-1 interact with $G\alpha_o$ and exhibit preference for the GDP-bound conformation. Psen-1 is primarily in the Golgi and endoplasmic reticulum, where it directly activates $G\alpha_o$ [161, 162]. The role of the interaction of these proteins with $G\alpha$ in the immune cells is unknown. Psen-1 mRNA transcripts are expressed in most cells of the innate and adaptive immune system (Tables 1–4)

6.7 Alternate heterotrimeric G-proteins GEFs

The involvement of the heterotrimeric G-proteins GEFs (NG-GPA, CSP, polycystin1 and polycystin like proteins) in the immune system is not known. However, they should be considered as these proteins principally regulate $G\alpha_i$ subunit activity and they are expressed in most of the cell types listed in Tables 1–4. A ligand-independent activator of heterotrimeric brain G-protein, yet to be characterized, was partially purified from detergent-solubilized extracts of the neuroblastoma-glioma cell hybrid NG108-15 and called NG-GPA (neuroblastoma-glioma G-protein activator) [163]. Cysteine string proteins (CSPs) are secretory vesicle proteins that are critical for neurotransmitter release. $G\alpha$ subunits interact with CSP in an ATP-dependent manner, while $G\beta\gamma$ subunits interact with CSP in both the

presence and absence of ATP. These interactions of CSP with both subunits of G proteins result in a tonic G protein inhibition of the N-type Ca^{2+} channels [164]. CSP also modulates G protein function by preferentially targeting the inactive GDP-bound form of $\text{G}\alpha_s$ and promoting GDP/GTP exchange [165]. Polycystin-1 is a large membrane-associated glycoprotein mutated in 85 % of autosomal dominant polycystic kidney disease (ADPKD), a major inherited disorder that is characterized by the growth of large, fluid-filled cysts from the tubules and collecting ducts of affected kidneys [166]. Biochemical analyses showed that C-terminal tail of polycystin-1 modulates Wnt signaling via stabilization of β -catenin [167] and activates the transcription factor AP-1 via c-Jun N-terminal kinase (JNK) and protein kinase C [168]. Polycystin-1 also binds and activates heterotrimeric G-proteins $\text{G}\alpha_i$, $\text{G}\alpha_q$, and $\text{G}\alpha_{12/13}$ and promotes activation of Jun kinases and AP-1 [169, 170]. The discovery of other members of polycystin 1 family, like polycystin1L2, has expanded the list of potential GEFs for $\text{G}\alpha$. It is reported to interact with $\text{G}\alpha_{12}$, $\text{G}\alpha_s$, and $\text{G}\alpha_{i1}$ and weakly with $\text{G}\alpha_{i2}$ [171].

7. CONCLUDING REMARKS AND QUESTIONS

The use of gene targeted mice models have created an explosion of data that has only begun to uncover the roles of heterotrimeric G proteins signaling in innate and adaptive immunity, and the consequences of disrupting these pathways for autoimmunity and infectious diseases. The discovery of the mechanisms by which heterotrimeric G protein can be activated independently of GPCRs will likely provide new insights into the way heterotrimeric G protein family members participate in immunity. Since many of the non-canonical G-proteins activators and regulators are broadly expressed, this will require the development of conditional, tissue-specific gene targeted mice to dissect their roles. Many areas of investigation remain open. For example, little is known about how the activities of the non-canonical GEFs and of their accessory proteins are regulated. What are the redundant versus specific functions for these proteins? Or otherwise reformulated, why such a diverse panel of activators and regulators? Given the diverse intracellular locations of heterotrimeric G-proteins and these regulatory proteins, functional roles at internal membranes such as phagosomes, endocytic vesicles, autophagosomes, mitochondrial membranes, and the nuclear membrane will likely be found.

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Highlights

1. Heterotrimeric G-proteins function in classical GPCR signaling.
2. Non-canonical G-protein signaling pathways exist.
3. Non-canonical G-protein signaling can intersect with classical GPCR signaling.
4. Non-canonical G-protein signaling pathways affect immune cell function.

Figure 1. Involvement of heterotrimeric Gα proteins in the immune system

Schematic representation of the immune system. Hematopoiesis originates from hematopoietic stem cells (HSC). Multipotent progenitors (MPP) give rise to common lymphoid (CLP) through early lymphoid precursor (ELP) or myeloid (CMP) progenitors. CLPs initiate the B/T cell lineages through early-thymic-progenitors (ETP) and pro-B cells (Fr. A), respectively. ETPs develop through CD4⁻CD8⁻ (DN) and CD4⁺CD8⁺ (DP) stages into mature T cells and Natural killer T (NKT) cells. In the bone-marrow, pro-B cells develop via pre-B (Fr. D) cells into immature B cells (Fr. E). These translocate into the

spleen to mature through transitional stages into mature B cells. Fetal liver HSC are responsible for the biogenesis of B1 cells. The ELP give rise to mature natural killer cells (mNK) found in the spleen generated in the bone marrow from progenitor (NKP) and immature (iNK). CMPs give rise to granulocyte–monocyte (GMP) and megakaryocyte–erythrocyte (MEP) progenitors and the indicated mature cell types.

Table 1
Relative mRNA expression of heterotrimeric G proteins and their known interacting partners in B cells

This table benefited from data assembled by Immunological genome project (<http://www.immgen.org/>)

		Cell type	B																Peritoneal Cavity	
		Organ	BM					Spleen												
		Subset	FrA	FrB	FrC	FrD	FrE	FrF	T1	T2	T3	Fo.	GC	MZ	B1a	B1b				
		Marker	Lin- AA4+Kb220 7Ra+B220	AA4+IgM+ 19a+3H 5A+	AA4+IgM+ 19a+3H 5A+	AA4+IgM+ 19a+3H 5A+	AA4+IgM+ 19a+3H 5A+	AA4+IgM+ 19a+3H 5A+	19a+IgM+ GLT+P 19+	19a+ IgM+ GLT+P 19+	19a+B220+ IgM+ GLT+P 19+	19a+B220+ IgM+ GLT+P 19+	19a+B220+ IgM+ GLT+P 19+	19a+B220+ IgM+ GLT+P 19+	19a+B220+ IgM+ GLT+P 19+	19a+B220+ IgM+ GLT+P 19+				
		Gene	Alternate name																	
G-protein family α-subunit	Gi	GNAO1		59	53	48	51	59	57	62	55	54	56	62	60	56	56			
		GNAI1		38	32	28	35	38	36	33	31	32	33	34	33	36	33			
		GNAI2		2834	3212	2940	3387	4063	3975	3975	4338	4229	3709	4343	3073	4394	4398			
		GNAI3		898	1189	1058	1074	917	930	1048	991	1129	1184	1129	789	1027	1069			
		GNA11		53	43	48	45	44	50	42	47	41	44	42	50	50	43			
	Gq	GNA12		65	55	65	55	56	62	60	59	53	55	63	58	69	60			
		GNA13		13	15	16	17	17	17	15	14	16	13	15	14	15	13			
		GNA2		49	49	41	48	45	48	45	41	44	40	130	39	41	42			
		GNA5		296	324	363	341	401	338	380	350	324	331	432	303	338	337			
		Golf	GNA1		21	18	21	22	18	23	20	21	23	24	20	19	20	19		
G-protein family β-subunit	Gβ1	GNAQ		323	304	277	247	248	160	249	288	283	192	193	248	215	284			
		GNAQ		202	249	406	382	283	180	206	197	207	193	167	228	224	224			
		GNA14		47	37	37	41	45	38	46	37	36	38	40	39	40	41			
		GNA15		318	211	66	71	109	135	89	101	97	129	90	80	56	72			
		GNA13		312	404	237	265	285	280	294	294	294	294	294	294	294	294			
	Gβ2	GNA13		871	767	780	829	1142	984	1205	1103	1034	1185	2078	760	874	850			
		GNA13		228	335	381	220	226	212	191	214	270	212	243	189	196	184			
		GNA13		822	887	868	844	905	766	800	783	775	800	847	924	864	864			
		GNA13		46	47	49	53	48	52	50	46	48	44	54	47	97	93			
		GNA13		79	106	117	77	66	56	68	60	55	48	195	49	41	50			
G-protein family γ-subunit	Gγ1	GNA13		60	54	56	48	54	51	47	41	43	46	54	55	52	52			
		GNA13		116	92	113	99	102	103	90	88	90	87	100	95	95	89			
		GNA13		3084	3197	3875	3397	3178	3048	3074	2818	2818	2875	3359	4387	4333	4183			
		GNA13		14	14	14	14	16	14	14	13	15	13	14	13	15	15			
		GNA13		150	334	372	343	381	272	414	369	344	312	448	388	205	253			
	Gγ2	GNA13		388	337	194	464	526	547	675	595	551	559	438	484	492	532			
		GNA13		40	35	39	45	40	38	35	38	37	33	50	37	39	36			
		GNA13		111	102	112	112	137	139	120	137	119	110	97	121	115	117			
		GNA13		265	228	234	170	138	181	210	198	209	254	296	250	239	221			
		GNA13		92	72	66	77	89	77	82	78	73	72	84	84	71	71			
RGS family	RGS1	GNG8		39	31	28	34	35	36	32	31	34	33	31	29	32	30			
		GNG10		561	513	414	264	363	479	394	453	475	424	571	564	629	667			
		GNG11		29	23	22	25	22	25	22	23	23	22	23	23	22	22			
		GNG12		619	238	274	216	187	168	201	173	160	147	593	142	291	282			
		GNG13		64	59	62	63	59	63	60	53	55	46	50	56	59	55			
	RGS2	RGS17		24	23	21	23	23	25	25	24	22	23	20	25	25	25			
		RGS19		463	387	319	457	778	755	874	767	786	786	654	698	725	690			
		RGS20		20	20	21	21	24	22	22	19	20	18	21	20	20	20			
		RGS21		202	390	318	534	690	74	289	170	236	108	462	86	66	66			
		RGS3		97	108	72	88	84	89	82	77	75	73	89	122	78	72			
RGS family R4/B	RGS4	RGS4		29	28	28	28	32	31	29	25	25	27	28	25	29	27			
		RGS5		117	17	19	21	22	19	20	20	18	17	19	19	21	20			
		RGS5		117	84	44	51	52	52	53	46	45	44	41	49	47	46			
		RGS13		10	10	10	11	19	10	15	18	61	11	844	10	11	12			
		RGS16		92	103	88	129	92	95	88	66	84	73	79	84	71	70			
	RGS18	RGS18		629	143	124	82	247	65	277	235	142	140	52	265	67	95			
		RGS21		13	14	14	14	15	13	14	12	14	12	13	12	13	13			
		RGS22		30	22	26	26	27	26	27	26	27	27	26	27	26	25			
		RGS23		23	19	20	20	24	22	22	22	21	21	21	22	23	24			
		RGS7		47	36	35	40	44	33	41	37	33	30	28	35	30	31			
RGS family R7/C	RGS8	RGS8		47	45	45	50	49	51	49	50	42	46	237	47	45				
		RGS11		60	46	40	52	60	56	56	48	50	52	52	55	52				
		RGS10		405	175	150	204	192	130	152	116	116	103	848	738	104	104			
		RGS12		213	271	405	268	120	84	90	71	76	69	92	74	67	64			
		RGS14		167	148	126	194	343	374	378	364	344	337	210	232	418	401			
	Group I (GEF)	AGS1		67	51	67	74	70	65	66	58	52	50	72	55	59	52			
		AGS3		216	186	183	181	154	145	130	130	138	125	92	123	104	125			
		AGS4		405	346	322	456	578	629	545	511	569	477	633	491	443	483			
		AGS5		68	128	351	120	60	45	41	36	33	39	307	33	40	36			
		AGS6		213	271	405	268	120	84	90	71	76	69	92	74	67	64			
AGS Group II (GPr)	AGS2	AGS2		31	28	31	31	34	30	34	34	27	30	28	32	32	31			
		AGS7		323	549	999	304	153	97	93	96	112	111	660	86	90	61			
		AGS8		38	29	33	38	37	35	35	34	34	32	33	31	34	32			
		Ric-8A		262	263	157	240	284	328	279	281	317	297	195	283	343	373			
		Ric-8B		233	244	269	303	317	253	369	411	336	336	280	347	254	315			
	Calnuc	RGS13		374	412	356	233	303	330	297	277	360	329	231	348	335	327			
		Nucb2		368	107	149	209	274	122	263	269	215	171	103	354	282	300			
		PKD1		205	149	155	149	190	324	218	266	271	317	130	507	291	344			
		PKD2L1		32	25	24	26	26	34	27	27	24	25	24	29	31	29			
		PKD2L2		349	291	366	366	366	364	418	418	462	418	339	377	343	391			
Accessory Gα protein	Dnaaj5	Dnaaj5		412	470	480	571	845	122	82	662	850	652	877	543	872				
		CCDC88a		144	167	188	125	118	59	88	90	80	75	88	32	31	51			
		APP		190	88	79	76	105	89	68	79	68	61	66	76	66	84			
		Psen1		414	461	458	504	520	479	565	520	531	564	579	653	597	617			
		GAP43		64	52	50	55	58	58	55	48	52	47	49	54	50	51			
	Pebp1	Tub1a		699	574	747	386	400	325	325	342	368	321	619	386	417	388			
		E2F3		706	672	885	686	385	363	323	353	375	541	1199	309	1615	901			
		Cav1		35	29	23	31	32	31	29	25	29	24	31	28	26	26			
		Pcp2		101	92	79	106	108	116	112	106	103	97	88	99	104	91			
		Pdc		20	24	18	22	21	23	20	20	19	20	22	20	19	19			
Receptor tyrosine kinase	Adrenk1	GRIN1		61	54	52	55	58	61	51	49	48	49	53	54	58	50			
		Hax-1		150	180	200	149	141	132	140	155	162	164	232	149	145	146			
		Hsp90		259	259	279	209	189	189	187	237	238	259	492	239	239	279			
		Tpr1		147	169	228	186	159	173	182	187	198	215	226	205	179	185			
		UNC5H2		61	52	53	55	69	60	60	54	53	53	51	61	55	61			
	Rag1/Gap	Rag1/Gap		52	44	39	48	46	52	48	45	43	38	42	46	37	52			
		FGFR3		31	40	40	40	40	40	40	40	40	40	40	40	40	40			
		FGFR		95	57	60	66	73	67	67	64	61	59	60	63	69	69			
		FGFR1		268	212	97	97	69	63	51	48	56	50	42	42	136	63			
		INSR1		13	23	24	24	24	24	24	24	24	24	24	24	24	24			
Receptor tyrosine kinase	CSF1R	INSR1		454	432	304	578	368	525	362	365	447	454	159	291	487	532			
		CSF1R		159	54	55	55	67	62											

Table 2
Relative mRNA expression of heterotrimeric G proteins and their known interacting partners in T cells

This table benefitted from data assembled by Immunological genome project (<http://www.immgen.org/>)

			Cell type	T																
			Organ	Thymus																
			Subset	preT ETP	preT ETP-2A	DN2	DN2A	DN2B	DN2-3	DN3A	DN3B	DN3-4	DN4	DP	4SP CD69+	4SP CD4int	4SP CD24-	8SP CD69+	8SP CD24int	8SP CD24-
			Marker	4-8-11b-11c-19-NK1.1-TCR-44b 117b-25b	4-8-11b-11c-19-NK1.1-TCR-44b 117b-25b	4-8-11b-11c-19-NK1.1-TCR-44b 117b-25b	4-8-11b-11c-19-NK1.1-TCR-44b 117b-25b	4-8-11b-11c-19-NK1.1-TCR-44b 117b-25b	4-8-11b-11c-19-NK1.1-TCR-44b 117b-25b	4-8-11b-11c-19-NK1.1-TCR-44b 117b-25b	4-8-11b-11c-19-NK1.1-TCR-44b 117b-25b	4-8-11b-11c-19-NK1.1-TCR-44b 117b-25b	4-8-11b-11c-19-NK1.1-TCR-44b 117b-25b	4-8-11b-11c-19-NK1.1-TCR-44b 117b-25b	4-8-11b-11c-19-NK1.1-TCR-44b 117b-25b	4-8-11b-11c-19-NK1.1-TCR-44b 117b-25b	4-8-11b-11c-19-NK1.1-TCR-44b 117b-25b	4-8-11b-11c-19-NK1.1-TCR-44b 117b-25b	4-8-11b-11c-19-NK1.1-TCR-44b 117b-25b	4-8-11b-11c-19-NK1.1-TCR-44b 117b-25b
			Gene	mRNA expression (A.U.)																
G-protein family α -subunit	GI	GIA1	54	56	54	45	53	56	61	60	58	67	52	63	64	58	48	55	55	
		GIA2	35	35	34	30	33	39	37	37	38	37	49	37	34	39	42	35	33	
		GIA3	2485	2434	2581	2413	2572	3384	2950	2260	1812	2377	3976	3699	2734	4490	3579	2636	2837	
		GIA4	945	1088	2078	1847	1848	1283	1298	1283	1448	1826	1144	1121	1972	1553	1148	1018	1018	
		GIA5	50	52	50	48	50	48	57	51	51	51	55	48	52	52	44	41	53	
	GII	GIIA1	63	64	63	60	59	66	59	73	62	59	74	61	64	59	52	49	49	
		GIIA2	14	14	14	13	13	14	14	14	15	13	24	14	16	16	16	14	14	
		GIIA3	43	42	43	39	44	45	44	47	54	46	68	49	58	46	58	42	48	
		GIIA4	317	323	352	360	352	368	309	318	283	343	231	312	312	314	338	346	354	
		GIIA5	25	24	24	24	23	23	21	20	24	22	30	24	22	20	26	23	21	
G-protein family β -subunit	GQ	GQIA1	292	314	228	274	235	166	200	144	135	115	83	98	95	73	75	99	70	
		GQIA2	163	168	230	202	238	235	278	269	203	261	170	196	197	232	210	202	272	
		GQIA3	57	65	45	42	42	39	40	39	39	40	56	41	44	42	36	36	39	
		GQIA4	89	97	96	75	62	100	129	92	96	91	89	80	80	73	81	73	72	
		GQIA5	438	589	648	478	377	566	433	363	265	304	164	254	244	257	287	263	276	
	G12/13	G12A1	282	234	148	180	72	78	38	37	36	37	59	42	42	38	44	34	37	
		G12A2	438	589	648	478	377	566	433	363	265	304	164	254	244	257	287	263	276	
		G12A3	981	885	714	952	1008	815	678	678	643	748	788	858	781	871	770	877	850	
		G12A4	291	279	511	347	412	482	333	780	561	561	535	594	548	388	437	435	370	
		G12A5	868	964	1108	992	963	955	1098	966	618	877	785	776	723	788	762	686	830	
G-protein-family γ -subunit	RZ/A	RZ/A1	50	49	48	44	48	60	50	51	51	54	55	51	48	47	44	51	48	
		RZ/A2	139	95	108	109	84	76	67	67	67	73	62	73	127	147	144	116	113	
		RZ/A3	64	58	61	55	65	60	59	59	63	62	68	60	63	66	64	60	65	
		RZ/A4	156	153	191	154	208	281	260	312	251	234	136	164	158	155	154	125	131	
		RZ/A5	14	15	15	15	16	14	15	14	17	15	20	13	13	16	15	14	14	
	R4/B	R4/B1	93	88	128	107	181	227	384	228	238	221	288	220	223	207	209	173	183	
		R4/B2	516	634	430	417	387	439	244	113	113	158	196	384	401	308	411	379	384	
		R4/B3	33	33	29	33	39	33	34	45	39	69	42	46	40	45	43	40	40	
		R4/B4	105	124	115	106	102	110	132	124	134	117	148	120	111	110	95	117	111	
		R4/B5	351	269	300	375	325	288	250	328	358	317	134	208	218	242	175	205	247	
RGS family	R7/C	R7/C1	69	97	96	75	62	100	129	92	96	91	89	80	80	73	81	73	72	
		R7/C2	34	36	32	35	33	35	34	32	38	32	40	34	34	32	38	31	32	
		R7/C3	659	544	646	758	514	381	351	445	417	428	234	332	352	369	297	256	340	
		R7/C4	25	24	25	22	24	24	24	24	24	24	24	24	24	24	24	24	24	
		R7/C5	403	397	377	455	417	295	190	246	267	238	181	232	269	205	160	168	128	
	R12/D	R12/D1	54	53	57	47	55	58	71	68	65	67	65	61	61	61	61	61	61	
		R12/D2	24	24	23	23	20	26	24	30	30	34	24	24	24	22	27	23	23	
		R12/D3	749	620	657	572	515	838	598	576	465	496	324	667	651	632	642	578	646	
		R12/D4	20	21	22	22	20	19	21	20	24	21	36	26	24	23	24	21	20	
		R12/D5	831	520	158	367	89	141	21	22	24	25	30	27	29	62	27	29	47	
AGS	Group I (GEF)	AGS1	810	683	392	330	416	300	237	196	233	213	135	136	122	118	122	89		
		AGS2	124	127	102	90	112	158	105	140	123	153	145	275	312	264	267	298	305	
		AGS3	26	28	25	23	26	23	29	28	33	29	58	23	27	27	33	25	14	
		AGS4	21	18	17	20	19	19	19	19	19	20	20	20	20	20	20	18	19	
		AGS5	75	66	45	42	48	53	52	48	47	45	53	49	50	56	48	49	45	
	Group II (GDI)	AGS6	9	9	11	9	9	11	10	11	10	12	11	10	10	10	10	9	9	
		AGS7	86	84	84	89	80	76	104	90	100	140	101	168	170	132	197	171	115	
		AGS8	1252	1372	774	893	457	265	69	17	16	15	21	15	15	14	16	14	16	
		AGS9	12	13	14	12	13	12	12	13	14	18	18	14	14	14	15	12	12	
		AGS10	22	27	25	23	26	23	29	28	30	33	35	27	30	26	30	24	27	
Accessory G α protein	Group III (Gpr)	AGS11	29	28	28	49	51	49	51	54	58	48	55	68	55	57	75	78	78	
		AGS12	472	273	273	242	218	216	432	432	432	432	432	432	432	432	432	432	432	
		AGS13	286	318	365	372	330	416	300	237	196	233	213	135	136	122	118	122	89	
		AGS14	95	103	88	81	90	116	146	166	167	231	419	449	443	485	369	331	388	
		AGS15	136	97	73	87	66	59	63	61	70	66	70	68	62	66	67	59	59	
	Hsp90	AGS16	255	280	227	285	249	230	233	220	161	174	140	147	147	117	161	155	143	
		AGS17	389	438	418	385	373	743	849	558	545	699	899	1067	1067	1067	782	673	688	
		AGS18	44	37	74	55	114	99	130	151	143	118	91	94	117	85	111	148	148	
		AGS19	266	318	365	372	330	416	300	237	196	233	213	135	136	122	118	122	89	
		AGS20	33	34	34	30	40	36	48	40	37	34	66	34	37	35	28	31	27	
Receptor tyrosine kinase	G12/13	AGS21	328	338	578	449	470	573	231	954	1019	787	220	93	92	157	173	234	177	
		AGS22	36	46	57	47	117	202	91	37	39	37	44	37	36	38	37	36	37	
		AGS23	439	387	282	352	315	300	268	188	143	171	230	231	233	253	235	224	245	
		AGS24	262	271	284	288	325	327	383	305	288	334	245	332	339	251	348	445	362	
		AGS25	793	839	848	674	778	1483	871	716	411	540	248	484	455	441	381	290	409	
	G12/13	AGS26	44	25	23	27	30	32	31	34	51	45	38	32	41	42	49	50	58	
		AGS27	238	257	211	215	207	231	312	222	168	184	232	271	258	311	293	298	346	
		AGS28	26	30	24	22	27	27	24	25	27	24	37	25	26	27	27	26	25	
		AGS29	439	489	438	438	438	374	489	284	372	325	325	325	325	325	325	325	325	
		AGS30	684	711	650	681	717	538	725	604	468	565	623	654	661	618	602	672	598	
Receptor tyrosine kinase	G12/13	AGS31	56	61	57	57	72	54	44	50	72	56	64	45	39	28	33	38	28	
		AGS32	71	64	69	53	59	82	74	57	66	59	68	70	69	123	76	63	104	
		AGS33	619	666	623	652	576	679	682	652	482	654	316	513	531	570	489	498	552	
		AGS34	53	58	53	54	46	45	54	58	66	59	92	61	58	62	55	50	52	
		AGS35	1284	1094	1594	1654	1314	1632	1822	1594	1138	1378	352	661	715	626	583	601	639	
	G12/13	AGS36	684	663	691	661	661	661	661	661	661	661	661	661	661	661	661	661	661	
		 				

Table 3
Relative mRNA expression of heterotrimeric G proteins and their known interacting partners in $\gamma\delta$ T, T, neutrophils, natural killer and NKT cells

This table benefited from data assembled by Immunological genome project (<http://www.immgen.org/>)

		Cell type	NKT										T										Neutrophils										NK																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																	
		Organ	Thymus			LN			Thymus			BM			Spleen			 		

Table 4
Relative mRNA expression of heterotrimeric G proteins and their known interacting partners in macrophages, monocytes and dendritic cells

This table benefited from data assembled by Immunological genome project (<http://www.immgen.org/>)

			Cell type	Dendritic cells										Macrophages and Monocytes									
			Organ	Spleen										BM	Spleen	Peritoneal cavity		BM		LN			
			Subset	CD4+	CD8+	triple negative	double negative	CD8-plasmacyt.	CD8+ plasmacyt.					Macr.	Red Pulp Macr.	Macr.	Classical Mono.	Non classical Mono.	Classical Mono.				
			Marker	11c+ B6- 4-11b+	11c+ B6- 4-11b+	11c+ B6- 4-11b+	11c+ B6- 4-11b+	11c+ B6- 4-11b+	11c+ B6- 4-11b+	11c+ B6- 4-11b+	11c+ B6- 4-11b+	11c+ B6- 4-11b+	11c+ B6- 4-11b+	B220- 3- Ly6C- 11b+ F4/80+	B220- 3- Ly6C- 11b+ F4/80+	B220- 3- Ly6C- 11b+ F4/80+	B220- 3- Ly6C- 11b+ F4/80+	B220- 3- Ly6C- 11b+ F4/80+	B220- 3- Ly6C- 11b+ F4/80+				
			Gene	11c+ B6- 4-11b+	11c+ B6- 4-11b+	11c+ B6- 4-11b+	11c+ B6- 4-11b+	11c+ B6- 4-11b+	11c+ B6- 4-11b+	11c+ B6- 4-11b+	11c+ B6- 4-11b+	11c+ B6- 4-11b+	11c+ B6- 4-11b+	B220- 3- Ly6C- 11b+ F4/80+	B220- 3- Ly6C- 11b+ F4/80+	B220- 3- Ly6C- 11b+ F4/80+	B220- 3- Ly6C- 11b+ F4/80+	B220- 3- Ly6C- 11b+ F4/80+	B220- 3- Ly6C- 11b+ F4/80+				
G-protein family α-subunit	G-protein family α-subunit	Gα	GNAO1	60	66	67	75	53	57	63	53	53	53	67	71	60	60	60	60	60			
			GNAA1	38	38	37	40	43	54	40	36	31	37	45	35	35	35	35	35	35			
			GNAB1	1048	1053	793	810	611	622	618	483	1344	692	477	635	635	635	635	635	635	635		
			GNAT1	52	53	59	53	47	47	52	47	45	64	60	50	50	50	50	50	50	50		
			GNAT2	53	66	49	58	53	47	78	47	15	17	15	16	16	16	16	16	16	16		
			GNAT3	15	14	19	16	16	14	14	15	14	15	14	15	14	15	14	15	14	15		
			GZ	50	48	56	67	44	41	66	51	44	59	62	56	56	56	56	56	56	56		
			GNA5	249	314	302	267	544	520	296	229	293	293	293	293	293	293	293	293	293	293		
			GNA6	22	19	21	27	22	21	21	21	21	21	21	21	21	21	21	21	21	21		
			GNA7	465	547	434	364	364	364	364	364	364	364	364	364	364	364	364	364	364	364		
			GNA11	157	146	137	151	523	524	221	147	221	243	242	242	242	242	242	242	242	242		
			GNA14	39	39	44	44	42	36	136	38	32	51	45	40	40	40	40	40	40	40		
G-protein family β-subunit	G-protein family β-subunit	Gβ	GNA15	53	208	115	105	493	511	358	323	263	353	372	317	317	317	317	317	317			
			GNA12	266	258	310	290	505	520	787	1249	500	564	492	317	317	317	317	317	317			
			GNA13	715	830	589	594	995	988	458	884	911	598	547	715	715	715	715	715	715			
			GNB1	134	112	134	140	136	150	275	160	115	146	146	146	146	146	146	146	146			
			GNB2	823	821	1061	801	842	854	1314	1314	1314	1314	1314	1314	1314	1314	1314	1314	1314			
			GNB3	50	47	52	56	38	40	49	51	61	58	54	54	54	54	54	54	54			
			GNB4	66	172	93	83	420	407	266	92	64	63	56	59	59	59	59	59	59			
			GNB5	63	64	62	82	51	51	85	46	81	74	69	68	68	68	68	68	68			
			GNB1L	97	88	122	138	97	88	162	90	106	130	143	101	101	101	101	101	101			
			GNB2L1	2696	3177	2684	2724	2696	2724	4849	4849	4849	4849	4849	4849	4849	4849	4849	4849	4849	4849		
			GNB2L2	15	14	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15		
			GNB2L3	961	159	459	590	314	298	460	651	345	523	1728	1728	1728	1728	1728	1728	1728	1728		
G-protein family γ-subunit	G-protein family γ-subunit	Gγ	GNB2	552	618	608	449	815	779	271	335	412	537	324	317	317	317	317	317	317			
			GNB3	41	46	37	54	34	34	42	37	33	49	50	41	41	41	41	41	41			
			GNB4	134	145	104	158	113	108	102	116	111	113	130	119	119	119	119	119	119			
			GNB5	378	335	475	383	291	265	406	346	691	435	403	705	705	705	705	705	705			
			GNB6	85	81	56	112	72	71	89	85	68	57	53	53	53	53	53	53	53			
			GNB7	35	37	37	38	33	33	32	33	34	39	42	36	36	36	36	36	36			
			GNB8	1289	1180	1528	1129	1585	1587	877	350	817	1878	877	938	938	938	938	938	938	938		
			GNB9	25	23	29	26	22	22	23	24	27	29	27	27	27	27	27	27	27	27		
			GNB10	140	402	236	196	826	767	578	486	526	511	406	368	368	368	368	368	368	368		
			GNB11	55	57	61	56	53	54	70	59	54	69	69	69	69	69	69	69	69	69		
			GNB12	140	402	236	196	826	767	578	486	526	511	406	368	368	368	368	368	368	368		
			GNB13	1289	1180	1528	1129	1585	1587	877	350	817	1878	877	938	938	938	938	938	938	938		
RGS family	RGS family	RGS	RGS17	26	27	26	29	20	22	24	20	22	27	29	27	27	27	27	27	27			
			RGS19	400	467	870	526	481	478	678	541	648	1168	540	540	540	540	540	540	540	540		
			RGS20	22	22	29	33	19	20	22	21	21	25	23	22	22	22	22	22	22	22		
			RGS21	2039	1167	1118	1183	258	260	60	625	378	26	23	378	378	378	378	378	378	378		
			RGS22	2045	1782	2088	2254	448	438	257	1818	579	696	843	1618	1618	1618	1618	1618	1618	1618		
			RGS23	227	116	105	222	54	74	100	248	74	84	218	85	85	85	85	85	85	85		
			RGS24	30	30	33	37	24	26	31	27	32	29	33	36	36	36	36	36	36	36		
			RGS25	21	26	24	20	20	19	19	18	19	21	24	22	22	22	22	22	22	22		
			RGS26	49	53	46	60	47	47	49	41	49	51	47	48	48	48	48	48	48	48		
			RGS27	9	10	11	11	10	10	10	10	12	13	18	18	18	18	18	18	18	18		
			RGS28	89	89	80	119	51	69	91	91	76	83	96	101	101	101	101	101	101	101		
			RGS29	102	328	798	454	1143	383	21	119	243	112	149	149	149	149	149	149	149	149		
AGS	AGS	AGS	RGS30	13	13	15	16	14	14	14	13	15	17	14	16	16	16	16	16	16			
			RGS31	28	28	29	30	27	27	30	28	27	29	30	30	30	30	30	30	30			
			RGS32	39	45	68	65	103	134	41	35	34	39	41	40	40	40	40	40	40			
			RGS33	54	53	45	59	43	41	48	49	54	51	49	49	49	49	49	49	49			
			RGS34	65	67	71	87	49	47	62	54	67	66	66	66	66	66	66	66	66			
			RGS35	640	1406	544	586	927	880	587	458	645	863	506	1090	1090	1090	1090	1090	1090	1090		
			RGS36	785	253	554	677	130	133	402	110	79	154	150	80	80	80	80	80	80	80		
			RGS37	202	181	183	241	263	253	253	253	253	253	253	253	253	253	253	253	253	253		
			Group I	AGS1	Dexras1	66	72	73	78	55	51	68	55	66	81	67	67	67	67	67	67		
			Group II	AGS3	GPSM1	120	123	149	167	147	146	196	92	133	116	171	103	103	103	103	103		
			Group III	AGS4	GPSM3, G18	643	605	743	769	473	470	442	422	551	744	770	605	605	605	605	605		
			AGS5	GPSM2, LGN	41	46	43	42	35	31	167	49	91	203	66	60	60	60	60	60	60		
Alternate GEFs	Alternate GEFs	Alternate GEFs	AGS6	RGS12	785	253	554	677	130	133	402	110	79	154	150	80	80	80	80	80			
			AGS2	Tctex-1	32	35	35	36	29	28	30	32	29	42	35	35	35	35	35	35	35		
			AGS7	TRIP13	128	111	166	117	46	57	424	75	151	398	238	65	65	65	65	65	65		
			AGS8	FNDC1	37	42	39	42	39	31	37	34	36	43	44	41	41	41	41	41	41		
			Ric-8A	196	208	223	220	306	307	282	192	237	297	334	166	166	166	166	166	166	166		
			Ric-8B	339	253	325	285	374	391	153	287	270	173	195	195	195	195	195	195	195	195		
			Nucb1	282	346	329	2	64	665	1469	390	824	1071	982	444	444	444	444	444	444	444		
			Nucb2	101	313	223	84	1981	1842	69	282	546	135	70	159	159	159	159	159	159	159		
			PKD1	224	231	192	212	407	474	228	310	268	258	265	194	194	194	194	194	194	194		
			PKD2L1	29	29	27	30	26	25	24	23	27	30	29	25	25	25	25	25	25	25		
			PKD2L2	620	463	464	458	402	422	154	707	450	143	131	289	289	289	289	289	289	289		
			Dnajc5	487	491	453	400	697	732	494	611	707	400	121	597	597	597	597	597	597	597		
CCDC88a	Gly, Girdin	783	756	695	724	537	591	57	633	646	85	74	545	545	545	545	545	545	545				
Accessory Gα protein	Accessory Gα protein	Accessory Gα protein	APP	1028	382	920	982	307	301		387	1972	4378	1769	794	1351	1351	1351	1351	1351			
			Psen1	604	703	533	435	794	803	684	629	960	1168	1061	615	615	615	615	615	615	615		
			GAP43	60	53	56	64	50	45	64	56	51	61	65</									