Normal Hematopoiesis and Inflammatory Responses Despite Discrete Signaling Defects in Gα15 Knockout Mice

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Received 1 November 1999/Accepted 4 November 1999

Gα15 activates phospholipase Cβ in response to the greatest variety of agonist-stimulated heptahelical receptors among the four Gq class G-protein α subunits expressed in mammals. Gα15 is primarily expressed in hematopoietic cells in fetal and adult mice. We disrupted the Gα15 gene by homologous recombination in embryonic stem cells to identify its biological functions. Surprisingly, hematopoiesis was normal in Gα15−/− mice, Gα15−/− Gqα−/− double-knockout mice (which express only Gα11 in most hematopoietic cells), and Gα11−/− mice, suggesting functional redundancy in Gq class signaling. Inflammatory challenges, including thioglycollate-induced peritonitis and infection with Trichinella spiralis, stimulated similar responses in Gα15−/− adults and wild-type siblings. Agonist-stimulated Ca2+ release from intracellular stores was assayed to identify signaling defects in primary cultures of thioglycollate-elicited macrophages isolated from Gα15−/− mice. Ca2+-stimulated phosphoinositide accumulation and Ca2+ release was significantly reduced in Gα15−/− macrophages. Ca2+ signaling was abolished only in mutant cells pretreated with pertussis toxin, suggesting that the C5a receptor couples to both Gα15 and Gα11 in vivo. Signaling evoked by other receptors coupled by Gq class α subunits appeared normal in Gα15−/− macrophages. Despite discrete signaling defects, compensation by coexpressed Gq and/or Gα11 α subunits may suppress abnormalities in Gα15-deficient mice.

Heterotrimeric G proteins transduce signals from ligand-activated seven-transmembrane domain receptors to effector proteins that regulate the release of intracellular second messengers, such as Ca2+ and cyclic AMP. A diverse family of G-protein-coupled receptors bind numerous hormones and neurotransmitters, peptides, small proteins, and lipid molecules. The biological functions mediated by G proteins are equally diverse, including behavioral and sensory functions, appetite control, arousal, metabolism, development, inflammation, and chemotaxis.

Heterotrimeric G proteins are composed of α and βγ subunits that can independently regulate effector proteins. Mammals express 16 distinct Gα subunit genes that are grouped in four classes, Gq, Gi, Gs, and G12, according to sequence similarity, effector regulation, and responsiveness to RGS (regulators of G-protein signaling) proteins, a recently identified family of GTPase-activating proteins (GAPs) for Gα subunits (30). The Gq class α subunits activate all isoforms of phospholipase Cβ (PLCβ), which hydrolyze the membrane lipid phosphatidylinositol 4,5-bisphosphate to produce inositol trisphosphate and diacylglycerol. PLCβ2 and PLCβ3 are also activated by Gβγ subunits, primarily released from Gi class G proteins (31), which is the basis for pertussis toxin inhibition of PLCβ activity and Ca2+ signaling evoked by Gi-coupled receptors (2, 20). Inositol trisphosphate produced by the activity of PLCβ evokes calcium release from intracellular stores, and diacylglycerol activates several isoforms of protein kinase C (PKC). Thus, Gq class α subunits regulate signaling pathways that are implicated in cellular proliferation and differentiation.

There are four Gq class α subunits in mice and humans; Gqα, Gα11, Gα14, and Gα15 (mouse or human ortholog, respectively [13, 33]). Gα15 and Gα11 are encoded by the tandemly duplicated Gna15 and Gna11 genes and co-localize to mouse chromosome 10, while Gqα and Gα14, encoded by the Gnaq and Gna14 genes, co-localize to mouse chromosome 19 (28). The two widely expressed Gq class α subunits, Gqα and Gα11, are 89% identical in amino acid sequence, and they couple an identical repertoire of receptors to PLCβ activation with similar efficiencies in vitro, in cultured cells, and in primary cells isolated from animals (28, 35). Analysis of single- and double-knockout (KO) mice with deficiencies in Gqα and/or Gα11 suggests that gene dosage may be a key factor in dissecting Gq class signaling pathways and their biological functions (28). The only apparent phenotypes of the single-KO Gqα−/− mice involve cell types or tissues, such as platelets and cerebellum, where Gα11, Gα14, and Gα15 expression is weak or absent (24, 27). Although mice with homozygous disruption of either the Gqα or Gα11 gene are viable and fertile, with only discrete phenotypic defects (24, 27), deletion of both genes (Gqα−/− Gα11−/−) results in embryonic lethality during midgestation (embryonic day 10.5) from a defect in cardiomyocyte proliferation (28). Cultured embryonic cardiomyocytes express Gqα and Gα11 but little or no Gα14 and Gα15, and in the absence of Gqα, Ca2+ signaling is no longer stimulated by the mitogenic factor angiotensin II (28).

The requirement of Gq class signaling in cell proliferation suggested a possible function of Gα15 in hematopoiesis. Gα15 is the most divergent of the Gq class α subunits (55% amino acid identity to Gqα, Gα11, and Gα14) and has the most restricted expression pattern, being principally confined to he-
matopoietic cells (Fig. 2 and reference 33). Though Gα15 activates PLCβ isoforms similarly to other family members, it possesses different pharmacological and biochemical properties in vitro (21). Gα15 couples to many receptors that are not activators of Gqq and Gα11 (26, 37) and therefore was promiscuous and Gαq and Gα15 properties in vitro (21). Gα15 possesses different pharmacological and biochemical properties.

FIG. 1. KO vector and strategies for characterizing homologous recombination by Southern blotting and genotyping by PCR after electroporation of the Gα15 replacement vector. (A) Wild-type (wt) allele and the targeting vector. The restriction sites are shown to scale. tk, thymidine kinase. (B) The KO allele. The pgk::neo insertion cassette is not drawn to scale. The SpeI, EcoRV, and HindIII sites used in characterizing the wild-type and KO alleles by Southern blot are shown on each allele. The inserted pgk::neo cassette is 2.1 kb in size and contains both EcoRV and SpeI restriction sites. The 5′ probe hybridizes to a 14-kb fragment in the wild-type allele and 9.5 kb in the KO allele when DNA is digested with EcoRV. The 3′ probe recognizes 9.2- and 7-kb fragments in wild-type and KO mice after digest with SpeI and HindIII. The Neo probe hybridizes to a 14-kb SpeI fragment in the KO allele. The relative positions of PCR products obtained from amplification of the wild-type allele with primers CT115 and CT133 (550 bp) and the KO allele with primers TW30 and TW144 (720 bp) are indicated. (C) Results obtained from genotyping Gα15+/−, Gα15+/−, and Gα15+−− tail DNA by Southern blot using the 5′ probe after digest with EcoRV; ethidium bromide-stained 2.5% agarose gel of PCR products obtained from amplifying the same tail DNA with the primers described above.

We used primary cultures of macrophages derived from the hematopoietic cells may also be controlled by other factors, such as RGS proteins, that could further limit the range of intrinsic compensatory mechanisms in G-protein signaling pathways.

MATERIALS AND METHODS

Knockout of Gna15. ES cells were maintained essentially as described previously (28). R1 ES cells from 129SV embryos were grown on primary embryonic fibroblasts rendered mitotically inactive by treatment with mitomycin. The Gna15 KO vector was linearized at a unique NotI site (Fig. 1) and electroporated into ES cells. Twelve heterozygous clones were obtained of 241 neomycin-resistant colonies. Four independent Gna15 heterozygous ES cell clones were injected into blastocysts, and one chimera gave germ line transmission of the mutant allele. Southern blots and 32P radiolabeling of probes were performed exactly as described elsewhere (13). Following extensive characterization of the ES clones and founder mice by Southern blot analyses to confirm homologous recombination and single integration of the targeting vector, genotyping was done by PCR. Oligonucleotide primers used were CT133 (CAGCAGCGCCAGCCTTAGTGATG) and CT115 (CTTTCGAGGAGGGATGCT), to amplify a 550-bp fragment in the wild-type allele, and TW30 (AGATGCGCATCATTCAAGCAGCCTCCTGTTCCAC), to amplify a 720-bp fragment in the KO allele (Fig. 1).

Preparation of membrane proteins for Western blot analysis of G-protein α subunits. Tissues were collected, and homogenates were prepared with a Dounce homogenizer in HMED (30 mM HEPEs, 2 mM MgCl2, 1 mM EDTA, and 1 mM dithiothreitol containing protease inhibitors (0.01 mg each of leupeptin and lima bean trypsin inhibitor per ml and 0.016 mg each of phenylmethyl...
sulfonamide, Na-p-tosyl-L-lysine chloromethyl ketone, and tosylsulfonyl phenylalanine chloromethyl ketone per ml. Homogenates were centrifuged at 500 x g to remove unbroken cells and nuclei, and supernatants were centrifuged at 100,000 x g. Membranes were suspended in HEMED, and protein concentrations were determined by Bradford assay using the Bio-Rad protein assay dye reagent concentrate prior to storage at -80°C. For sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), 5 μg of protein was measured and loaded per lane unless specified. Antibody B861 recognizes specifically the C-terminal end of Gα15, and antibodies W082 and B825 recognize specifically Gαq and Gα11, respectively.

Fluorescence-activated cell sorting. Peripheral blood was collected from the tail vein of Alsever’s solution (Gibco-BRL, Grand Island, N.Y.), and single-cell suspensions were prepared from hematopoietic tissues for antibody analysis. Conjugated anti-mouse antibodies were used to B220-biotin, CD4-fluorescein isothiocyanate, CD8-biotin, Gr-1-biotin, Mac-1-biotin, and Mel-14-fluorescein isothiocyanate; streptavidin-phycocerythrin (Pharmingen, San Diego, Calif.) was used when applicable; 5,000 events were collected per sample on a FACScan analytical instrument (Becton Dickinson Co., San Jose, Calif.).

Bone marrow transfer and growth. Single-cell suspensions were prepared from bone marrow of wild-type and KO donor mice and was used for growth studies in MethoCult M-3430 (StemCell Technologies Inc., Vancouver, British Columbia, Canada) or for bone marrow transfer. Recipient mice (BALB/c) were exposed to two doses of irradiation, 500 and 400 rads, in a Gamma Cell 40 small-animal irradiator containing two 280 kV sources (Atomic Energy Ltd., Ottawa, Ontario, Canada). Irradiated recipient mice were injected with 5 x 10^6 donor cells in the lateral tail vein. Splenocytes were collected from recipient mice 9 days later and fixed in Bouin’s fixative. Spleen suspensions (CFU-S) were counted in a ZN2-6 tissue culture microplate. In a first experiment, four recipient mice were injected with medium alone, eight were injected with wild-type cells, and eight were injected with Gα15-/- cells. In a second, independent experiment, four recipient mice were injected with medium 10 were injected with wild-type cells, and 10 were injected with Gα15-/+ and Gα15-/-.

Thioglycolate-induced peritonitis. Mice between 8 and 12 weeks of age were injected with 1 ml of 3% thioglycolate. Peritoneal cavities were lavaged at 0 (uninjected), 18, 48, or 96 h following intraperitoneal (i.p.) injection of thioglycolate. Cells from the peritoneal lavage fluid were spun onto a slide with a cytospin. Cells were loaded with Fura-2/AM in the presence of 10% glycerol, 1% Triton X-100, 100 mM NaF, 0.2 mM sodium orthovanadate, 100 ng/ml with the starvation medium. Cells were stimulated with 100 nM C5a or 2 x 10^-7 M (1) The PI assays were performed with 100 nM C5a in the Gα15-/+-mediated assay. The numbers of CFU-S present on the spleens of lethally irradiated mice were determined by plating 48 h after injection and stained with Diff-Quik. The proportion of eosinophils in 100 to 200 leukocytes was determined for each smear.

Thioglycolate-elicited macrophages for ex vivo experiments and macrophage cultures. C57BL/6 wild-type or Gα15-/- old mice were perfused in the peritoneal cavity. Cells from the peritoneal lavage fluid were spin onto a slide with a cytopsin. Slides were stained with Diff-Quik (Fisher Diagnostic). Two to seven mice of each genotype were analyzed at each time point. Differential counts of neutrophils, eosinophils, and mononuclear cells were determined with a 100 x oil immersion lens. A minimum of 100 cells were examined at least three times per preparation.

Trichinella spiralis infection. Twelve-week-old mice were administered 500 infective larvae orally. Six mice of each genotype were analyzed. Blood smears were taken from the tail veins in duplicate at days 6, 9, 11, 13, 15, 17, 21, 23, 25, and 30 after infection and stained with Diff-Quik. The proportion of eosinophils in 100 to 200 leukocytes was determined for each smear.

Fura-2 Ca2+ imaging. Cells were plated on coverslips and processes as described elsewhere (36). Cells were loaded with Fura-2/AM in the presence of Pluronic for 20 min and then set on ice. Coverslips were mounted onto perfusion chambers and cells were continuously perfused during fluorescence recording. Fluorescence was recorded with the Delta Scan imaging system and an IC-200 camera. The images were captured with the Image Master program and analyzed with Felix.

PI assay. Cells were distributed to 12-well tissue culture dishes at 10^6 cells per well and metabolically labeled 48 h with 8 μCi of [3H]thymidine (DuPont) per ml in labeling medium (inositol-free DMEM, 5% FBS) 4 days after injection. Cells from two to three mice were pooled per genotype. Macrophages were purified by adherence to the tissue culture dish.

Results

The Gα15 gene (Gα15) was disrupted in mice by homologous recombination in ES cells. The replacement vector (Fig. 1A) contained a neomycin resistance gene under the control of the PGK promoter (pgk::neo) flanked by 5’ (5-kb) and 3’ (3.4-kb) segments of Gα15. A null allele was created by homologous recombination of the replacement vector to delete 8 kb that included exons 3 through 6 of Gα15. The deleted exons contained three of the five amino acid motifs (7) which are essential for guanine nucleotide binding to the α subunit. The targeting vector was electroporated into ES cells, and heterozygous clones were obtained under selection with Geneticin and ganciclovir. Heterozygous ES cell clones were identified with the 5’ probe and confirmed with the 3’ probe and the Neo probe. Nine chimeric mice were obtained, and one transmitted the Gα15 null allele to offspring. Intercrossing heterozygous Gα15+/− mice produced viable and fertile homozygous null (Gα15−/−) progeny (Fig. 1C).

Gα15−/− mice did not exhibit obvious phenotypic defects. Western blotting using an antibody directed at the C-terminal residues of Gα15 confirmed that a null allele was created (Fig. 2B). Gα15 was detected in membrane proteins from cell types and tissues that normally express Gα15 in wild-type mice but not in those isolated from Gα15−/− mice. To assess whether expression of other Gq class α subunits was altered in the absence of Gα15, we also assayed expression of Gαq, Gα11, and Gα14 in the same tissues by Western blot analysis (Fig. 2B and data not shown). The relative levels of expression of each of these G-protein α subunits were similar in wild-type and Gα15−/− mice.

Hematopoiesis appears normal in single and double Gq class KO mice. Differential expression of Gα15/Gα16 in hematopoietic cells according to their maturation stage (2, 16, 32, 33) suggested that hematopoiesis might be altered in Gα15−/− mice. Therefore, we characterized the cellular composition of isolated bone marrow cells from Gα15+/− mice by FACScan analysis. Single-cell suspensions were prepared for analysis from peripheral blood, spleen, thymus, peripheral and mesenteric lymph nodes, Peyer’s patches, and bone marrow isolated from wild-type or mutant mice. No differences between Gα15−/− and wild-type mice were observed in the tissue composition of B cells, CD4+ and/or CD8+ T cells, neutrophils, and monocytes. The architecture of the major lymphoid organs, including thymus, spleen, peripheral lymph nodes, and Peyer’s patches, was likewise intact. The proportion of plasma cells was normal as determined by hematocrit (wild type, 49.8 ± 0.003%; Gα15−/−, 50.9 ± 0.013).

The integrity of the erythroid and myeloid precursors of the Gα15−/− bone marrow was assessed by bone marrow transfer to lethally irradiated recipient mice (erythroid lineage) and by bone marrow culture in methylcellulose (myeloid lineages). The numbers of CFU-S present on the spleens of lethally irradiated recipient mice 9 days after injection with bone marrow cells were similar in recipients rescued with Gα15+/− (11.4 ± 4.4) or Gα15−/− (8.6 ± 3.5) cells. Growth in methylcellulose also indicated that there was no difference in the number and ability of myeloid precursors to proliferate and differentiate in response to the growth factors provided in the methylcellulose (data not shown). Our findings that Gα15−/− mice produce normal numbers and ratios of B, T, and myeloid cells suggest that Gα15 is not required during hematopoiesis.
To address the potential compensatory role of other Gq family members, mice deficient in both Goq and Go15 were assessed for hematopoietic competence. Double-KO Goq−/−Go15−/− mice were obtained by crossing single-KO mice with null mutations in the Goq and Go15 genes. The double KO mice have all the features of the Goq−/− mice single-KO mice (24, 27); they are smaller than their littermates and exhibit the same bleeding disorder and ataxia. These defects are not altered by the simultaneous absence of Go15 and Goq. As had been observed in the Go15−/− mice, FACScan analysis showed that proportions of B cells, T cells, granulocytes, and monocytes in all tissues tested were not significantly different from those observed in wild-type animals. In a bone marrow transfer experiment, the erythroid precursors of the Goq−/−Go15−/− bone marrow were fully competent to rescue lethally irradiated recipient mice. The number of colonies on the spleens of recipient mice injected with Goq−/−Go15−/− bone marrow (13.5 ± 1.5) was not significantly different from the number of CFU-S found in recipients of wild-type cells (11.3 ± 3.8). No obvious hematopoietic deficiencies were detected in any of the Gq class single-KO mice, possibly indicating that Gq class signaling is not required in steady-state hematopoiesis. How-
macrophages, in agreement with the results obtained in the Ca^{2+} fluorescence assay. In contrast, the responses to UTP and PAF (Fig. 4A and B) increased for at least 5 min after stimulation and were similar in macrophages from wild-type and Ga\(\alpha\)15\(^{-/-}\) mice. The macrophages were treated with pertussis toxin for 24 h at 100 ng/ml to determine if the remaining activity observed in the Ga\(\alpha\)15\(^{-/-}\) cells could be attributed to Gi coupling. The PI response to C5a was completely eliminated in Ga\(\alpha\)15\(^{-/-}\) macrophages in the presence of pertussis toxin (Fig. 4C). PI release in response to C5a in the wild-type cells was reduced minimally by 50% in the presence of pertussis toxin. These results suggest that the C5a receptor (C5aR) is normally coupled to both Ga\(\alpha\)15 and Gi class G proteins and that the signal observed in the macrophages in response to C5a is therefore probably due to remnant Gi stimulation. The response to PAF was diminished approximately 40% by pertussis toxin in both wild-type and Ga\(\alpha\)15\(^{-/-}\) cells, while the response to UTP was reduced 30% by pertussis toxin in wild-type and Ga\(\alpha\)15\(^{-/-}\) mice (Fig. 4B). These results suggest that as with the C5aR, the UTP and PAF receptors couple to Gi G proteins, but in contrast to the C5aR, Ga15 does not exclusively mediate the Gq component of Ca^{2+} signaling in macrophages. The different patterns of G-protein coupling by these receptors suggests that the normal expression of Gi and/or Gq proteins may compensate for Ga15 deficiency and thus explain the absence of a more obvious biological phenotype in the Ga15 KO mice.

**ERK activation by C5a is normal in Ga15\(^{-/-}\) macrophages.** Agonist stimulation of the C5a receptor was shown to activate the mitogen-activated protein (MAP) kinase pathway in a pertussis toxin-sensitive manner in human neutrophils (8) and in transfected cells (9). Because we observed a synergistic effect of Gi and Ga15 on PI release in response to C5a in wild-type and Ga15\(^{-/-}\) macrophages, we addressed the possibility of a similar effect on ERK activation. Wild-type and Ga15\(^{-/-}\) macrophages treated with or without pertussis toxin were stimulated with 100 nM C5a for 3 min. The activation of ERK1 and ERK2 was assessed by Western blotting. The V667A antibody (Promega) recognizes only the active, dually phosphorylated ERKs. The activation of ERKs in response to C5a was rapid, equivalent in wild-type and Ga15\(^{-/-}\) cells, and completely inhibited by pertussis toxin treatment in both wild-type and Ga15\(^{-/-}\) macrophages (Fig. 4D). These data indicate that C5a-induced ERK activation is entirely dependent on Gi signaling in murine macrophages, as it is in mouse and human neutrophils (8).

**DISCUSSION**

G proteins mediate responses to a multitude of signaling molecules that evoke neuronal, hormonal, and sensory signal transduction as well as inflammatory and immune responses. The inflammatory response to destroy foreign particles and pathogens requires an intimate interaction between numerous...
cell types that stimulate cell proliferation and differentiation and activate leukocyte motility and chemotaxis to the site of infection. Chemokines and classical chemoattractants stimulate these processes and are implicated in autoimmune diseases (reviewed in reference 29). Many of these agonists, including IL-8, C5a, and formylmethionyl-leucyl-phenylalanine, bind heptahelical receptors to active PLCβ and evoke Ca²⁺ release. A number of these chemokine receptors are coupled by pertussis toxin-sensitive Gi class proteins and Gα₁₅, the pertussis toxin-insensitive Gq class subunit that is predominantly expressed in hematopoietic cells (3, 22).

Several features of Gα₁₅ made it an interesting target for mutational analysis in mice. A wide variety of heptahelial receptors which were initially found to activate Gs or Gi class α subunits were later found to also couple Gα₁₅ and Gα₁₆ (mouse and human orthologs, respectively), but not Gαq/11, to the activation of PLCβ. This suggested that Gα₁₅ may have unique functions, independent of Gαq/11, in hematopoietic tissues where it is normally expressed. Gα₁₅ exhibits the most restricted expression pattern of any G-protein subunit, with the exception of those expressed only in specialized sensory transduction cells. Gα₁₅ also has evolved at an accelerated rate relative to all other mammalian α subunit genes (13). Many genes whose primary function serves hematopoietic cell types have been noted to diverge at an accelerated rate relative to other members of the same gene family (23). This may reflect selection pressures on Gα₁₅ and other genes which are specifically expressed in the immune cells that defend against pathogens (23). Therefore, although Gα₁₅ is always coexpressed with Gαq and Gα₁₁, we anticipated that it would have unique functions in hematopoiesis or immune cell function. Not only are the Gα₁₅⁻/⁻ mice viable and fertile, but Gα₁₅ does not appear to be required for hematopoiesis. This result was surprising since Gα₁₅ is abundantly expressed in murine erythroleukemia cells (33), and antisense expression of Gα₁₆ reportedly inhibited cellular growth rates of erythroleukemia cells in culture (15). By contrast, we observed normal production of plasma erythrocytes in Gα₁₅⁻/⁻ adult mice, and we did not observe an increase in fetal mortality which could have been indicative of a deficiency in fetal erythropoiesis. Furthermore, Gα₁₅ is abundantly expressed in pre- and pro-B-cell lines and in the bone marrow (16, 33), which is rich in B cells, myeloid and lymphoid precursors, and mature neutrophils. However, all hematopoietic lineages, including B cells, T cells,
neutrophils, and monocytes, appeared to be normal in the Go15−/− mice, as did the architecture of the major lymphoid organs. Minimally, we anticipated a perturbation in the ratios of these cell-types in Go15−/− mice, as was observed in the IL-8 receptor (IL-8R) knockout mice, because IL-8R is coupled by Go15 (but not Goq or G11) to the activation of PLCβ (34). The IL-8R−/− mice exhibited neutrophil and B-cell expansion resulting in lymphadenopathy, splenomegaly, and foci of hematopoiesis in the liver (11). Our analysis of Go15−/− mice indicates either that Go15 does not couple the IL-8R in vivo or that compensatory mechanisms allow IL-8 signaling in the absence of Go15.

Analysis of Gq class KO mice indicates that Gq11 signaling is subject to gene dosage effects (28), consistent with their similarities in receptor coupling and effector activation (35). Goq and G11 are widely expressed throughout development and in adult mice. Homozygous deficiency of either gene can be tolerated, and the phenotypic abnormalities in Goq−/− and Go11−/− mice are relatively mild. By contrast, double-KO (Goq−/−G11−/−) mice die during midgestation (embryonic day 11) due to cardiomyocyte hypoplasia and subsequent heart failure (28). Addition of a single active allele of either Goq or G11 allows fetuses to survive until shortly after birth, and addition of two active gene copies, one of each or two of either gene, allows mice to survive to adulthood and to reproduce. The most obvious phenotypes in Goq−/− mice, ataxia and a bleeding disorder (24, 27), are not apparent in either Go11−/− or Goq−/−G11−/− mice. These phenotypic differences may result from the fact that Goq is more widely expressed and is more abundant than G11 in the affected tissues, although molecular mechanisms of signaling specificity have not been rigorously tested. We reasoned that gene dosage effects may be revealed in Go15−/− Goq−/− mice. The ataxia and bleeding disorder previously found in Goq−/− mice were not enhanced in the double-KO mice, nor were additional defects in hematopoiesis detected. Go11 is the only Gq class α subunit remaining in most hematopoietic cells in Go15−/− Goq−/− mice. To explain the absence of phenotypic defects in the double-KO mice, and assuming that Gq class signaling is important in hematopoietic cells, either Go11 conveys all Gq class activity during hematopoiesis in normal mice or these Gq proteins are functionally redundant and Go11 compensates for the absence of Go15 and/or Goq in the double-KO mice. The former possibility appears unlikely because the Go11−/− single-KO mice have no apparent hematopoietic defect (data not shown). Unfortunately, the Go11−/− Go15−/− double-KO mice cannot be obtained by crossing the single-KO strains due to their chromosomal colocalization and analysis of triple mutants will require conditional KO technology because the Goq−/− Go11−/− mice die in utero before initiation of hematopoiesis in the fetal liver. It is therefore not yet possible to determine the function of Gq class signaling in hematopoiesis.

We next tested the possibility that Go15 mediates signaling during immune challenge. The chemokine receptors expressed on leukocytes mediate inflammatory responses and the redundancy of pathogen receptors have been shown in transfusion assays to couple to Gi class α subunits and Go15 but not Goq or G11 (14, 22, 34). We used thioglycolate-induced peritonitis to survey the response of neutrophils and mononuclear cells to a non-antigen-specific, T-cell-independent agent (thioglycolate). Infection with T. spiralis was used to monitor the eosinophil response to a challenge that required recognition of specific antigens. The Go15−/− mice performed normally in these and several other challenges not described, such as ovalbumin-induced eosinophilia, croton oil-induced dermatitis, and turpentine-induced fever. A normal response to all tests was observed in Go15−/− mice despite the fact that defective responses to an inflammatory challenge with thioglycolate occurred in chemokine receptor (e.g., IL-8R and CCR2) KO mice as well as in the PLCβ2 KO mice (5, 11, 19). Thus, the approach of using systemic readouts such as hematopoiesis or immune challenges, which are regulated by multiple signaling mechanisms, failed to detect a deficiency in Go15−/− mice.

Many cell types and signaling pathways which may mask a deficiency in Go15 are engaged during inflammation. We reasoned that signaling defects in isolated cells might identify physiologically relevant pathways coupled by Go15. Therefore, we challenged purified thioglycolate-elicited macrophages of wild-type and Go15−/− mice with different G-protein-coupled agonists and measured activation of PLCβ in single cells. We found three agonists, the anaphylatoxin C5a, UTP, and PAF, that stimulated G-protein-dependent Ca2+ signaling (Fig. 3 and 4). Go15−/− macrophage stimulation with C5a exhibited diminished inositol phosphate production and Ca2+ release compared with wild-type cells (Fig. 3 and 4C). By contrast, UTP and PAF stimulated similar responses in mutant and wild-type macrophage. Our studies are in agreement with previous analysis of the C5a response in cultured cells cotransfected with CsAR and G16, which suggested that CsAR-evoked Ca2+ signaling was mediated by Go15 but not Goq11 (1, 9). However, pertussis toxin inhibition of PLCβ was more pronounced in the macrophage from Go15−/− mice than in either study. Additionally, transfected cells apparently required both Gi and Go15 coupling to the CsAR for full activation of the MAP kinase (10). By contrast, activation of MAP kinase was completely dependent on Gi-mediated signaling in both mouse macrophages (Fig. 4) and human neutrophils (8).

C5a is an 8.6-kDa terminal by-product of complement activation with inflammatory properties. Mice with an homozygous disruption of the CsAR (CsAR−/−) have several inflammatory phenotypes. They are sensitive to pulmonary infection with Pseudomonas aeruginosa and are resistant to the reverse-passive Arthus reaction (immunocomplex-induced granuloma formation) in the lung, skin, and peritoneum (18). Go15 may be involved in mediating these effects. However, due to the ability of the receptor to induce a PI signal and Ca2+ release in the absence of Go15, the remaining signal observed may be sufficient to produce a full biological response.

In a previous study, the Ca2+ response evoked by UTP was entirely and specifically blocked by the expression of antisense Go16 RNA in the HEL human erythroleukemia cell line (4). In the same study, pertussis toxin partially inhibited UTP-mediated signaling in the parental cells, suggesting a synergistic activity between Go16 and βγ released from Gi. However, we found that UTP-evoked PI production and Ca2+ release appeared normal in macrophages isolated from Go15−/− mice (Fig. 4). Thus, the UTP responsive P2Y2 receptor expressed in murine macrophages can apparently be coupled by Gi and Gq/11. The PAF receptor is similarly coupled by Gi and Gq/11 in Go15−/− macrophages.

The ex vivo experiments with peritoneal macrophages suggest that the absence of an apparent phenotype in Go15−/−, Go11−/−, and Go15−/− Goq−/− mice may be explained by the ability of the receptors that mediate hematopoiesis and inflammatory responses to couple to multiple G proteins, including those of both the Gi and the Gq class.

ACKNOWLEDGMENTS

ES cell lines (R1) were graciously provided by J. Rossant. We thank M. J. Bennett, G. Spangrude, and S. Muullem for insightful comments and for help with bone marrow transfers and Ca2+ imaging. Antibodies were kindly given to us by P. Sternweis, S. Mumby, and M. Cobb.
This work was supported by Pharmacological Sciences Training Grant 5-T32-GM07062 (I.D.) and by National Institutes of Health grant DK47890, March of Dimes, Leukemia Association of North Central Texas, Texas Advanced Research Program, and an American Heart Association Established Investigator Award (T.M.W.).

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