Because T cell differentiation leads to an expanded repertoire of chemokine receptors, a subgroup of G protein-coupled receptors, we hypothesized that the repertoire of G proteins might be altered in parallel. We analyzed the abundance of mRNA and/or protein of six G protein α-subunits in human CD4⁺ and CD8⁺ T cell subsets from blood. Although most G protein α-subunits were similarly expressed in all subsets, the abundance of Gαo, a protein not previously described in hematopoietic cells, was much higher in memory versus naive cells. Consistent with these data, activation of naive CD4⁺ T cells in vitro significantly increased the abundance of Gαo in cells stimulated under non-polarizing or T11-17 (but not T11-1 or T11-2)-polarizing conditions. In functional studies, the use of a chimeric G protein α-subunit, Gαqα5, demonstrated that chemokine receptors could couple to Gαo-containing G proteins. We also found that Gα1i, another α-subunit not described previously in leukocytes, was expressed in naive T cells but virtually absent from memory subsets. Corresponding to their patterns of expression, siRNA-mediated knockdown of Gαo in memory (but not naive) and Gα1i in naive (but not memory) CD4⁺ T cells inhibited chemokine-dependent migration. Moreover, although even in Gαo- and Gα1i-expressing cells mRNAs of these α-subunits were much less abundant than Gα12 or Gα13, knockdown of any of these subunits impaired chemokine receptor-mediated migration similarly. Together, our data reveal a change in the repertoire of Gαi/o subunits during T cell differentiation and suggest functional equivalence among Gαi/o subunits irrespective of their relative abundance.

Unlike naive cells, which are generally homogeneous except for their antigen receptors, the effector/memory population is highly heterogeneous. For CD4⁺ or helper T (T(h)1)³ cells, separate pathways of effector/memory differentiation have been described, resulting in, for example, T(h)1, T(h)2, and T(h)17 cells, which can be identified by their abilities to produce the signature cytokines, interferon-γ (IFN-γ), interleukin 4 (IL-4), and IL-17, respectively. For CD8⁺ or cytotoxic T cells, progressive differentiation results in the production of proteins such as perforin and granzymes, which enhance the ability of these cells to kill infected cells (1–3).

A more general scheme has been proposed for understanding CD4⁺ and CD8⁺ effector/memory T cell differentiation, whereby naive cells give rise first to central memory T cells (T(CM)), and these can in turn, either through homeostatic or antigen-driven proliferation, yield effector memory T cells (T(EM)) (4) (not to be confused with effector/memory cells). T(CM) express L-selectin (CD62L) and the chemokine receptor CCR7, which are necessary for trafficking to non-inflamed lymphoid tissues, whereas T(EM) lose expression of CD62L, CCR7, or both along with the ability to enter non-inflamed lymphoid organs. T(EM) possess the highest levels of effector functions, such as the production of effector cytokines or cytotoxicity (4). An expanded ability to migrate into tissue to combat infection is an essential component of effector/memory T cell differentiation. This latter capability is due in part to the expression of an expanded number of species of chemokine receptors (for example, from two on naive cells, to at least 15, in various combinations, on effector/memory T cells) and is associated with an intrinsic, enhanced response to chemokines in assays in vitro (5). In humans, chemokine receptors form a group of 19 seven-transmembrane domain, G protein-coupled receptors (GPCRs), which have ligands that form a corresponding group of more than 40 chemoattractant proteins.

We hypothesized that the expanded repertoire of receptors on memory T cells might be associated with gains and/or losses of downstream effector molecules, such as the heterotrimeric G proteins, which are the proximal transducers of chemokine receptor signals. Changes in the abundance of G protein α-subunits have functional consequences for GPCR-mediated signaling. In some cellular contexts, the abundance of certain G protein α-subunits is limiting, and increased responsiveness to various ligands is achieved through the increased expression of

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9 The on-line version of this article (available at http://www.jbc.org) contains supplemental Figs. S1–S3.

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9 The abbreviations used are: T(h), helper T (cells); IL, interleukin; T(EM), central memory T cells; T(EM), effector memory T cells; GPCR, G protein-coupled receptor; NP, nonpolarized; TCR, T cell receptor; rIL, recombinant interleukin.
**Goα Expression in Human Memory T Cells**

G protein α-subunits, as well as, or in place of, increased expression of a given GPCR (6–10). Regulation can be subtle, for example the result of selectively increasing or decreasing the abundance of individual Goα-family members while leaving others unchanged (7, 8), and changes in the abundance of various G proteins can result in functional differences by affecting the balance among different signaling pathways (6). Changes in the abundance of Gi-family G proteins might have particular relevance for chemokine receptor signaling, because these G proteins are critical for chemoattractant receptor signaling (11–13).

We compared naive and memory T cells for the expression of G protein α-subunits. In experiments described below, we found that effector/memory T cells from human peripheral blood, much more so than naive T cells, expressed the Gi family member Goαi, which has not been described previously in hematopoietic cells. In functional studies, we found that despite being of relatively low abundance in the effector/memory population based on mRNA expression, Goαi contributed significantly to chemokine receptor signaling in these cells. We also found that Goαi had a reciprocal pattern as compared with Goαo, Goαi was expressed and functioned with chemokine receptors in naive cells but was down-regulated after differentiation to effector/memory cells. Together, these data are the first description of the presence and function of Goαi (and Goαo) in leukocytes and suggest that the low abundance of some Go proteins belies their functional importance in GPCR signaling.

**EXPERIMENTAL PROCEDURES**

**Cell Culture and Reagents**—Cell culture media and salt solutions were obtained from Invitrogen, and fetal bovine serum (FBS) was purchased from Gemini Bio-Products (Woodland, CA). Ficoll–Hypaque was purchased from Amersham Pharma- cia Biotech. All recombinant chemokines were purchased from Peprotech (Rocky Hill, NJ). The anti-CD3 mAb (OKT3) was obtained from Ortho Biotech (Raritan, NJ). The anti-CD28 mAb clone 9.3 was a kind gift from Carl June (University of Pennsylvania, Philadelphia). Anti-IL-4, anti-IL-12, and anti-IFNγ antibodies were obtained from BD Biosciences. rIL-2 was obtained from Hoffmann-La Roche. The cytokines IL-4, IL-12, IFNγ, and TGF-β were purchased from R&D Systems (Minne apolis, MN). Rabbit polyclonal antibodies against Goα12 (catalogue number sc-7276), Goαo (sc-262), Goαi (sc-387), Goαq (sc-392), Goαδ (sc-410), goat polyclonal antibody against actin (sc-1615), and anti-rabbit and anti-goat preadsorbed HRP-conjugated secondary antibodies were purchased from Santa Cruz Bio technology, Inc. (Santa Cruz, CA). A second rabbit polyclonal antibody against Goαi (catalogue number 3975) was purchased from Cell Signaling Technology (Danvers, MA). The metalloproteinase inhibitor TAPI-0 was obtained from Peptides International (Louisville, KY).

**Plasmid DNAs**—cDNA encoding human CXCR6 was amplified by polymerase chain reaction (PCR) using the previously described pCEP4:CXCR6 clone C3-9.1 as a template (14) and inserted in-frame into the pEYFP-N1 vector (Clontech Laboratories, Palo Alto, CA) to encode a CXCR6 C-terminal fusion protein with yellow fluorescent protein (YFP). DNAs were verified by sequencing. The construction of plasmids expressing G protein chimeras, in which the five C-terminal amino acid residues of Goαq were replaced with those of Goα12 (q5 chimera), Goαo (q5s), or Goαi (q5s), has been described previously (15, 16); these were a kind gift of Bruce M. Conklin (Gladstone Institute of Cardiovascular Disease, University of California, San Francisco). All chimeras have an internal hemagglutinin (HA) epitope, allowing for recognition by an anti-HA antibody (clone 12CA5, Roche Diagnostics). The cDNA clones for human Goαi1, Goαi2, Goαi3, GoαoA, GoαoB, and Goαi3 in pcDNA3.1(+), were obtained from the University of Missouri cDNA Resource Center.

**Purification and Sorting of Leukocyte Subsets**—Elutriated lymphocytes and monocytes were obtained from healthy donors by the Department of Transfusion Medicine, Clinical Center, National Institutes of Health (Bethesda, MD), under an Institutional Review Board–approved protocol. In initial experiments, CD4+ and CD8+ T lymphocytes were isolated from elutriated lymphocytes by negative selection with either the CD4+ T cell isolation kit II or the CD8+ T cell isolation kit (Miltenyi Biotec, Auburn, CA) according to the manufacturer’s instructions. These cells were further purified using CD45RA microbeads (Miltenyi Biotec) for positive and negative selection of naive and memory cells, respectively. CD45RA−CD4+ T cells (memory T cells) were further purified by incubation with CD62L microbeads for positive selection of central memory (CD62L+) and negative selection of effector memory (CD62L−) cells. For the isolation of other leukocyte subsets from elutriated cells, positive selection was performed using microbeads for the following cell type–specific markers (all from Miltenyi Biotec): CD3 (total T cells) and CD11b (monocytes). All selected leukocyte subsets were analyzed for phenotypic cell surface marker expression by antibody staining and flow cytometric analysis as described below. In later experiments (indicated in the legends to Figs. 2, 3, and 8), CD4+ and CD8+ T cell subsets were first purified by negative selection with either the RosetteSep CD4+ T cell or CD8+ T cell enrichment mixtures (StemCell Technologies, Vancouver, British Columbia, Canada) as described previously (17), and naive and effector/memory subsets were purified by sorting using a FACSAria flow cytometer (BD Biosciences) after staining with the following antibodies obtained from BD Biosciences: FITC-conjugated anti-CD8, phycoerythrin-conjugated anti-CD62L, phycocerythrin-Cy5-conjugated anti-CD3, allophyocyanin-conjugated anti-CD45RO, and allophyocyanin-Cy7-conjugated anti-CD4 as described previously (18). It is noteworthy that published experiments have demonstrated the cleavage of CD62L from the surface of lymphocytes through the activity of a zinc metalloproteinase (19, 20). In our sorting of CD8+ T cell subsets, we sometimes detected substantial amounts of CD62L mRNA in the “CD45RO−CD62L−” effector/memory population, presumably through contamination with naive cells in which CD62L had been cleaved. We found that treatment of CD8+ T cells during staining and sorting with TAPI-0 (50 μg/ml), a specific inhibitor of the zinc metalloproteinase responsible for CD62L cleavage (21), resulted in very low amounts of CD62L mRNA in the effector/memory population (data not shown). The data presented here are from experi-
ments in which real-time RT-PCR analysis demonstrated little or no contamination of the CD8^+CD62L^− subsets with cells expressing mRNA for CD62L (data not shown).

Flow Cytometry—For phenotypic analysis of leukocyte subsets based on the expression of cell surface markers, 100 µl of cells at 1 × 10^7 cells/ml in PBS, 1% FBS, 10 mM HEPES was incubated with dye-conjugated primary antibodies (1 µg) for 15 min on ice. Cells were then washed twice with PBS/FBS/HEPES and fixed in 1% paraformaldehyde. Antibody-staining data were collected on a FACSCalibur (BD Biosciences) flow cytometer. Flow cytometry data files were analyzed with FlowJo software (Tree Star Inc., San Carlos, CA).

In Vitro Activation of Naive Cord Blood CD4^+ T Cells—Human cord blood was obtained from term placentas following the delivery of healthy newborns at Shady Grove Adventist Hospital (Gaithersburg, MD) as approved by that institution’s review board. Naive CD4^+ T cells were isolated from cord blood with the RosetteSep/human CD4^+ T cell reagent (StemCell Technologies). Stimulation of the CD4^+ T cells was performed as described previously (17). Cells were cultured at 1 × 10^6 cells/ml in 24-well plates in RPMI 1640 medium/10% FBS with gentamicin (20 µg/ml; Invitrogen) and stimulated with plate-bound anti-CD3 (OKT3, 10 µg/ml), soluble anti-CD28 (1 µg/ml), and rIL-2 (200 IU/ml). In addition, these cells were treated under one of four regimens: anti-IL-4 (0.4 µg/ml), anti-IL-12 (2 µg/ml), and anti-IFNγ (8 µg/ml) with rTGF-β (10 ng/ml) for nonpolarizing (NP) conditions; rIL-12 (2 ng/ml) and anti-IL-4 (0.4 µg/ml) for T_{h1}-polarizing conditions; rIL-4 (4 ng/ml), anti-IL-12 (2 µg/ml), and anti-IFNγ (8 µg/ml) for T_{h2}-polarizing conditions; and with no cytokines (other than rIL-2) and no antibodies as a control. On day 3 or 4, cells were harvested, washed, and resuspended in fresh medium at 1 × 10^6 cells/ml containing rIL-2 and the appropriate cytokine and antibody combinations. In some experiments, to produce T_{h17} cells, CD4^+ T cells from cord blood were activated with anti-CD3 and anti-CD28 as above (without rIL-2) with rIL-23 (100 ng/ml), rIL-6 (20 ng/ml), rIL-1β (10 ng/ml), rTNFα (10 ng/ml), and rTGF-β (either 2 or 10 ng/ml) as well as anti-IL-4 (0.4 µg/ml), anti-IL-12 (2 µg/ml), and anti-IFNγ (8 µg/ml) antibodies.

Isolation of RNA, Synthesis of cDNA, and Semiquantitative Real-time RT-PCR—In early experiments, total cellular RNA was isolated with the TRIzol reagent (Invitrogen), and first-strand cDNA synthesis was performed with the SuperScript first-strand synthesis system for RT-PCR (Invitrogen) as described by the manufacturer. In experiments involving cells purified by cell sorting, RT-PCR was performed with the Platinum quantitative inventoried RT-PCR ThermoScript one-step system (Invitrogen) as described by the manufacturer. Invented primer and probe sets (FAM/MGB-labeled) for human Gα_{o1}, Gα_{o2}, Gα_{o3}, Gα_{o3}, Gα_{o3}, CD3, CD62L, and GAPDH and for mouse Gα_{o2}, Gα_{o2}, and β_2-microglobulin were purchased from Applied Biosystems (Foster City, CA). Primers and probes specific for human Gα_{o2} and Gα_{o2} were designed with Primer Express v2.0 (Applied Biosystems) with probes overlapping variant-specific splice junctions. Primer/probe sequences are available upon request. Real-time PCR analysis was performed on samples in duplicate with either an ABI 7700 or 7900 se-
**Gαo Expression in Human Memory T Cells**

**FIGURE 1.** Gα₀ is expressed in peripheral blood T cells, preferentially in the memory subset. A, CD4⁺ T cells were isolated from peripheral blood by negative selection and then further purified into naïve and effector/memory CD4⁺ T cells. Whole-cell lysates (100 μg of protein) were analyzed by Western blotting with rabbit polyclonal anti-Gα₀ or anti-actin antibodies as indicated. Results are for naïve and effector/memory CD4⁺ T cells from one donor and are representative of three donors. Representative full-length Western blots that show molecular mass markers and positive controls can be seen in supplemental Fig. S1. B, CD3⁺ and CD3⁻ cells were purified from peripheral blood with magnetic beads for CD3. Whole-cell lysates (100 μg of protein) were then analyzed by Western blotting with anti-Gα₀ and anti-actin antibodies. Results are for cells from one donor and are representative of two donors. C, CD3⁺ and CD3⁻ cells were isolated as described for B, total RNA was isolated from each subset, and samples were then analyzed by semiquantitative real-time RT-PCR for the expression of mRNAs for Gα₀, Gα₁₂, CD3, and as a control, GAPDH. The fold-differences in abundance of the indicated mRNAs, each normalized to GAPDH expression, between CD3⁺ (gray bars) and CD3⁻ cells (black bars) were calculated according to the method of ∆∆Ct. For each mRNA of interest, the lowest level of expression versus GAPDH from a single well for both CD3⁺ and CD3⁻ samples was set at one. Values cannot be compared between mRNA species. The data shown are the mean ± S.E. in expression for the indicated mRNAs from one donor from assays performed in duplicate and are representative of three donors, separate from those analyzed in B. **, p < 0.005; and ***, p < 0.0005, when comparing CD3⁺ and CD3⁻ cells. D, poly(A)⁺ mRNA from mouse and human brain and spleen samples were analyzed by semiquantitative real-time RT-PCR for the expression of mRNAs for Gα₀ and either β₂-microglobulin in the case of mouse samples or GAPDH for human samples. Data shown are the mean ± fold-differences ± S.E. in Gα₀ mRNA for each sample within each species, assayed in triplicate, from one experiment.

**Chemotaxis Assays**—Chemotaxis assays were performed according to standard protocols. Cultured cells were washed twice in PBS, counted, and resuspended in chemotaxis medium (RPMI 1640 containing 25 mM HEPES, pH 8, and 1% BSA) at 10⁶ cells/ml. Chemotaxis assays were performed with Transwell plates containing 5-μm pores. Cells in 100 μl of chemotaxis medium were added to inserts that were preincubated at 37 °C for 30 min before being placed in wells that contained either medium alone or medium with 100 ng/ml CXCL12 or CCL20 and incubated for 2 h at 37 °C, 5% CO₂. Cells that had migrated into the lower wells were harvested and counted with a Vi-CELL analyzer (Beckman Coulter). Dose-response experiments showed that 100 ng/ml was the optimal concentration for both CXCL12 and CCL20 in these chemotaxis assays (data not shown).

**Western Blotting**—For analysis of leukocyte populations for the expression of individual G protein α-subunits, total protein was measured using either the Bio-Rad assay or Pierce Coomasie Plus reagent (Pierce Biotechnology, Inc.). Cell samples containing equal amounts of protein were boiled in 2× Laemmli buffer and resolved by 10% SDS-PAGE. We used gels of both 8.6 × 6.8 cm and 16 × 16 cm. Gels were transferred to Immobilon-P PVDF membranes (Millipore Corp., Bedford, MA) overnight in the cold in a Transblot apparatus (Bio-Rad Laboratories) at 50 V in 25 mM Tris, 190 mM glycine, 20% methanol. Blots were washed in Tris-buffered saline, pH 7.4, containing 0.01% Tween-20 (TBST) and then blocked in TBST containing 2% (w/v) nonfat dry milk (Bio-Rad Laboratories) for 1 h at room temperature. All subsequent incubations and washes were done in TBST, 2% milk. Blots were incubated with the appropriate primary antibodies (at 1:200 dilution, or 1 μg/ml) for 2 h followed by two 10-min washes and then incubated in a 1:2000 dilution of the appropriate secondary antibody (HRP-conjugated goat anti-rabbit, or donkey anti-goat) for 30 min followed by two washes. For visualizing bands, blots were treated with SuperSignal West Pico chemiluminescent substrate (Pierce Biotechnology) and exposed to X-OMAT XAR film (Eastman Kodak Co.). Quantification of Western blots was performed with Adobe Photoshop (San Jose, CA) and ImageJ software (National Institutes of Health) as described previously (25).

**Statistical Analysis**—Unpaired, two-tail t tests were performed using InStat version 3 software from GraphPad Software Inc. (San Diego, CA). Statistical significance was indicated by a two-tailed p value of <0.05. Where multiple comparisons were required, simple one-way ANOVA was performed followed by running the Bonferroni post-hoc test.

**RESULTS**

**Gα₀ Is Expressed in Human Peripheral Blood T Cells**—To detect differences in the expression of G proteins between naive and effector/memory T cells, we analyzed purified human peripheral blood CD4⁺ T cells by Western blotting with subtype-specific antibodies against a number of G protein α-subunits. The major G₁ family members found in lymphocytes, Gα₁₂ and Gα₁₃ (26, 27), were equivalently abundant in naïve and memory cells, as were Gα₁₁ (Fig. 1A) and Gα₁₃ (Fig. 1A). Because the G₁ proteins are crucial to chemokine-induced functions in leukocytes (28), we investigated the expression of Gα₀, a G₁ family member for which the expression and function in leukocytes had not been described previously. (In the absence of a reliable specific antibody, we were unable to assess the expression of...
that the anti-Gαi1 protein.) We could easily detect Gαi1 protein but only in effector/memory cells (Fig. 1A). Representative full-length Western blots that show molecular mass markers and positive controls can be seen in supplemental Fig. S1A. In supplemental Fig. S1B, using transfected cells, we provide additional evidence that the anti-Gαi1 antibodies recognize the appropriate proteins. We also confirmed our finding that the Gαi1 protein was expressed in effector/memory CD4+ T cells in separate experiments using a second commercially available anti-Gαi1 antibody (supplemental Fig. S2).

This finding led us to examine other leukocyte populations for the expression of Gαi1. Analysis of Western blots of lysates of cells purified from peripheral blood on the basis of CD3 revealed that Gαi1 was found in CD3+ cells (consisting mainly of CD4+ and CD8+ α/β T cells) but not in CD3- cells (Fig. 1B). This difference was confirmed at the level of mRNA by real-time RT-PCR analysis of the same cell types, where the relative difference in the expression of Gαi1 mRNA between the purified CD3- and CD3+ samples was similar to that of CD3 itself (Fig. 1C). The abundance of Gαi1 mRNA was not significantly different between the groups (Fig. 1C).

The experimental advantages of using mice led us to investigate whether Gαi1 was found in mouse lymphocytes, where published data suggested that Gαi1 was not expressed (29). To compare relative levels of Gαi1 mRNA, we analyzed the abundance of Gαi1 mRNA in spleen and brain for both BALB/c mouse and human tissues. In the human samples, real-time RT-PCR analysis showed a ΔΔCt value of between 6 and 7 when comparing brain and spleen RNAs, indicating an ~90-fold higher abundance of Gαi1 mRNA in brain compared with spleen (Fig. 1D). However, Gαi1 mRNA, although highly expressed in mouse brain, was undetectable in spleen, resulting in a ΔΔCt of at least 25 (Fig. 1D), consistent with published data from Northern blot analysis (29). Together, the data demonstrate that although Gαi1 can be detected in human T cells, this is not the case in mice.

Gαi1 Is Most Abundant in Differentiated CD4+ and CD8+ T Cells—Because we found that Gαi1 protein was found in CD4+ T cells and that it was more abundant in memory than in naive CD4+ T cells, we looked more carefully at Gαi1 mRNA and protein in specific subsets of CD4+ and CD8+ T cells, which were sorted on the basis of the surface markers CD62L and CD45RO to define naive (CD62L+CD45RO-), central memory (CD62L-CD45RO-), and effector memory (CD62L-CD45RO+; CD62L+CD45RO+) cells (5, 18). CD45RO is a protein-tyrosine phosphatase involved in T-cell activation for which alternative mRNA splicing yields isoforms differentially expressed on naive cells compared with effector/memory cells (30). Substantial numbers of CD62L-CD45RO- cells are present only within the CD8+ population; we needed to use the metalloproteinase inhibitor TAPI-0 to obtain pure populations of these cells, which otherwise were contaminated.

**FIGURE 2.** Gαi1 mRNA and protein expression is highest in the most differentiated subsets of human CD4+ and CD8+ T cells. CD4+ (A) and CD8+ (B) T cells from peripheral blood were sorted into naive (CD62L+CD45RO-), TCM (CD62L+CD45RO-), and TEM (CD62L-CD45RO-; CD62L+CD45RO+) subsets as described under “Experimental Procedures.” Each subset was analyzed by semiquantitative real-time RT-PCR for mRNAs for Gαi1, Gαi1, Gαi1, and Gαi1. -Fold differences in the abundance of the indicated mRNAs, each normalized to that of GAPDH mRNA, between each subset of CD4+ (A) or CD8+ (B) T cells were calculated for each mRNA species as described in the legend for Fig. 1. For each mRNA of interest, the lowest level of expression versus GAPDH from a single well was set at 1. Values cannot be compared between mRNA species. The data shown are the mean ± fold differences ± S.E. in expression for the indicated mRNAs from assays performed in duplicate from separate donors for CD4+ and CD8+ T cells and are representative of five and three donors for CD4+ and CD8+ T cells, respectively. *, p < 0.05, when compared with mRNA in naive subsets; #, p < 0.05 when comparing mRNA content of the CD62L+CD45RO- subset with that of the CD62L-CD45RO- subset. C, whole-cell lysates (40 μg of protein) of purified subsets of CD4+ and CD8+ T cells, isolated as described under “Experimental Procedures,” were analyzed by Western blotting with anti-Gαi1 and anti-actin antibodies. Results are for naive and combined memory subsets (CD4+ Mem, CD8+ Mem) of CD4+ or CD8+ T cells. Data are from the same donor for both CD4+ and CD8+ T cell subsets and are representative of three donors.
with naive (CD45RO−) cells that had lost surface CD62L during processing (see “Experimental Procedures”). The three subsets of CD4+ and four subsets of CD8+ T cells were sorted by flow cytometry, and real-time RT-PCR was performed as described previously. Analysis of CD4+ T cell subsets revealed that, as before, Gaα mRNA was expressed at low abundance in naive cells (CD62L+CD45RO−) but was progressively more abundant in TCM (CD62L−CD45RO−) followed by TEM (CD62L−CD45RO−) (Fig. 2A). Moreover, none of the mRNAs for Gaα, Gaβ, and Gaγ were significantly different in their abundance among the CD4+ T cell subsets.

A similar pattern of Gaα mRNA expression was observed when sorted subsets of CD8+ T cells were analyzed (Fig. 2B). Naive CD8+ T cells expressed the lowest amount of Gaα mRNA, whereas the most differentiated TEM (CD62L−CD45RO−) generally had the highest abundance of Gaα mRNA, with no significant differences among the subsets for the other G protein α-subunits analyzed (Fig. 2B). We analyzed purified subsets of CD8+ T cells and, for the purposes of comparison, CD4+ T cells from the same donor by Western blotting. Memory cells were pooled for Western blotting because of limitations in the numbers of cells within memory subsets from individual donors. Consistent with the above patterns of Gaα mRNA expression, Gaα protein was more abundant in memory CD8+ T cells than in naive cells (Fig. 2C). Both naive and memory subsets of CD8+ T cells had higher amounts of Gaα protein than did their CD4+ T cell counterparts (Fig. 2C), although Gaα could not be detected in naive CD8+ T cells from all donors (data not shown).

Gaαβ mRNA Is More Abundant Than Gaαα mRNA in Differentiated T Cells—Alternative splicing of Gaαα mRNA gives rise to two protein isoforms, Gaαα and Gaαβ, which differ in their C-terminal sequences. The Gaαα isoform is more abundant than the Gaαβ isoform in mouse brain and heart (29, 31, 32). We used real-time RT-PCR to examine the relative expression of Gaαα and Gaαβ mRNAs in T cell subsets. We first used plasmid DNAs encoding Gaαα or Gaαβ sequences to demonstrate the specificities and equal efficiencies for the primer/probe sets that we had designed (data not shown).
Gαo mRNA was ~10-fold more abundant than GαqA mRNA in human spleen (Fig. 3C).

Gαo mRNA Expression Is Increased in CD4+ T Cells Activated in Vitro under Nonpolarizing or TH17-polarizing Conditions—The increased expression of Gαo mRNA in TEM compared with naive cells suggested that Gαo expression was induced during T cell activation and differentiation. We investigated this with an in vitro system for activation and differentiation of human T cells. Cord blood naive CD4+ T cells were activated in the presence of cytokines and anti-cytokine antibodies to yield T11, T12, or NP cells, and cells were also activated in the presence of IL-2 alone, i.e., under “neutral” conditions without other added cytokines or anti-cytokine antibodies. Consistent with our previous data from peripheral blood (Figs. 1A and 2C), naive CD4+ T cells from cord blood had no detectable Gαo by Western blotting (Fig. 4A). Activation of these cells for 4 days led to an increase in the abundance of Gαo protein in cells that were not polarized but in cells activated under T11- or T12-polarizing conditions (Fig. 4A). Analysis of Gαo mRNA by real-time RT-PCR supported the Western blotting data (Fig. 4B). By contrast with Gαo, the amounts of mRNAs for Gα12, Gα13, and Gα13 were not affected by activation under these conditions (Fig. 4D).

In an attempt to examine the stability of Gαo expression in non-polarized cells, we activated naive cord blood cells as described above, and after 6 days removed the cells from activation and cultured them for 3 days in IL-2 alone. A sample of nonpolarized cells was also moved to media with polarizing cytokines, either IL-12 or IL-4. After the 9 days, Gαo protein was still confined to the non-polarized cells and was not detectable in cells activated initially under either T11- or T12-polarizing conditions (Fig. 4C, Day 9 Activated). Treatment of nonpolarized, Gαo-high cells with IL-12 for 3 days substantially decreased the abundance of Gαo whereas treatment with IL-4 had no such effect (Fig. 4C, 3d cytokines). In investigating non-T11 and non-T12 subsets; we next analyzed cord blood cells cultured under conditions that have been described as driving mouse CD4+ T cells to differentiate to produce IL-17 (33). As noted above, T11 cells are a recently recognized separate lineage of non-T11, non-T12 memory/effector CD4+ T cells thought to be critical in autoimmune disease (34). Cells activated under T11 conditions showed significant induction of Gαo mRNA, comparable to that seen in the nonpolarized cells (Fig. 4D).

Chemokine Receptors CCR6, CXCR6, and CXCR4 Couple to Gαo-containing G Proteins—We next investigated whether the presence of Gαo in memory/effector T cells might have functional relevance for the many chemokine receptors found on these cells. We measured intracellular Ca2+ mobilization in cells transfected with plasmids encoding chimeric G protein α-subunits, in which the last five amino acid residues of Gαo have been replaced with those of Gαo, Gαq3, or Gα13 to create the chimeras Gαq5, Gαq5, and Gαq5, respectively (15, 16, 35). Because the last five amino acid residues of the α-subunit are critical for the coupling of a G protein to a given GPCR, Gαq5, Gαq5, and Gαq5 allow GPCRs coupled to Gαo, Gαq2, and Gαq3-containing G proteins, respectively, to activate Gαq-dependent pathways such as phospholipase Cβ-mediated intracellular Ca2+ mobilization (15, 16, 35). Because the last five amino acid resi...
dues of \( \alpha_{oA} \) and \( \alpha_{oB} \) are identical (29), the \( \alpha_{q5} \) chimera serves to analyze responses common to both isoforms.

Transfection of an HEK293 cell line stably expressing CCR6 with a plasmid encoding \( \alpha_{q5} \) resulted in an increase in the mobilization of intracellular \( \text{Ca}^{2+} \) in response to the CCR6 ligand, CCL20, when compared with cells transfected with empty vector (Fig. 5A), similar to the increase following transfection with a plasmid encoding \( \alpha_{q5} \) (Fig. 5B). CCR6 is a

FIGURE 5. The human chemokine receptors, CCR6, CXCR6, and CXCR4 can couple to G proteins containing the chimeric \( \alpha_{q5} \). A–C, HEK293:CCR6 cells were transfected with plasmids encoding each of the chimeric G protein \( \alpha \)-subunits, \( \alpha_{q5} \) (A), \( \alpha_{q5} \) (B), and \( \alpha_{q5} \) (C), and as a control, the empty vector, pcDNA3.1(+), as described under “Experimental Procedures.” D–F, in separate experiments, HEK293T cells were co-transfected with plasmids encoding CXCR6-YFP, each of the chimeric G protein \( \alpha \)-subunits, \( \alpha_{q5} \) (D), \( \alpha_{q5} \) (E), and \( \alpha_{q5} \) (F), and as a control, the empty vector, pcDNA3.1(+). G–I, Jurkat T cells, which express CXCR4 endogenously, were transfected with plasmids encoding each of the chimeric G protein \( \alpha \)-subunits, \( \alpha_{q5} \) (G), \( \alpha_{q5} \) (H), and \( \alpha_{q5} \) (I), and as a control, the empty vector, pcDNA3.1(+). After 48 h, cells were harvested, loaded with fura-2/AM, and assayed for intracellular calcium mobilization in response to treatment with the chemokine CCL20 (for CCR6), CXCL16 (for CXCR6), or CXCL12 (for CXCR4) at 1 μg/ml, added at –25 s. Fluorescence measurements were made as described under “Experimental Procedures.” Individual panels show intracellular calcium mobilization for cells transfected with a plasmid encoding the G protein chimera (black trace) compared with pcDNA3.1(+)-transfected control cells (gray trace) from one experiment each for HEK293:CCR6 cells (A–C), HEK293T cells (D–F), and Jurkat cells (G–I) and are representative of three experiments.
receptor found on effector/memory T cells (36) and, in particular, on all cells of the T17 lineage (37–39). As expected, given that chemokine receptors do not couple to G proteins (28), transfection with Goq5s had no effect on Ca2+ mobilization (Fig. 5C). Western blots of cell lysates showed equivalent abundance of all three HA-tagged chimeric G proteins (data not shown). We performed similar experiments in 293T cells co-transfected with a plasmid encoding YFP-tagged CXCR6, a second chemokine receptor found on effector/memory T cells (40), and either control empty vector or plasmids encoding the chimeric G protein α-subunits as described above and treated with the CXCR6 ligand, CXCL16. As for CXCR6, CXCR6 coupled to Goq6s (and Goq15s) but not Gq5s-containing G proteins (Fig. 5, D–F). Analogous experiments were performed in transfected Jurkat T cells, a human CD4+ T cell line, in which we found that the endogenously expressed chemokine receptor CXCR4, which is expressed on both naive and memory T cells, could also couple to both Gq5s and Gq5s-containing G proteins to mobilize intracellular calcium (Fig. 5, G–I). The effects of expressing Gq5s or Gq15 on signaling through CXCR6 and CXCR4 were smaller than for CCR6, possibly because co-transfections were used to express CXCR6 and because of the lower efficiency of transfection for Jurkat versus HEK293T cells.

Knockdown of Goq Inhibits CXCL12-dependent Chemotaxis and Ca2+ Flux—To determine whether the presence of Goq in T cell subsets was physiologically relevant, we used siRNA to knock down Goq in Jurkat cells and examined these cells for their ability to migrate or mobilize intracellular Ca2+ in response to CXCL12. Knockdown of Goq resulted in a ~40% decrease in the number of cells that migrated to CXCL12 compared with that of cells transfected with either control siRNA or siRNA specific for Goq5, which is not expressed in these cells (Fig. 6A). We confirmed these data by knocking down Goq with an independent Goq-specific siRNA (23) (supplemental Fig. S3). Surprisingly, the effect of knockdown of Goq on chemotaxis was similar to that of knockdown of either Gao12 or Gao13 (Fig. 6A). Chemotaxis of cells transfected with siRNAs against both Goq and Gao13 was even more significantly inhibited than that of cells depleted of either protein alone. The efficiencies and specificities of these siRNAs in knocking down their targets were verified by Western blotting (Fig. 6B). We also examined the effect of knockdown of these Gα subunits on CXCL12-dependent Ca2+ mobilization, which is a much more rapid functional response to stimulation of chemokine receptors than is chemotaxis. Knockdown of Goq, Gao12, or Gao13 had similar effects on the inhibition of CXCL12-induced Ca2+ flux as compared with cells transfected with either control or Goq-specific siRNAs (Fig. 6C). Consistent with our chemotaxis data (Fig. 6A), knockdown of both Goq and Gao12 had a more dramatic effect on Ca2+ flux than that observed in cells transfected with either siRNA alone (Fig. 6C).

We extended our findings by examining the effect of knockdown of G protein α-subunits in primary cells. We sorted naive and memory subsets of human CD4+ T cells from peripheral blood and transfected them with the appropriate siRNAs. Transfection of naive CD4+ T cells with siRNA against Goq had no effect on their chemotaxis to CXCL12 (Fig. 7A), consistent with their lack of expression of Goq. However, knockdown of Gao12 reduced CXCL12-mediated chemotaxis by ~35% compared with that of control cells (Fig. 7A). In contrast, knockdown of Goq in memory CD4+ T cells had a similar effect to that of Gao12 knockdown on chemotaxis to CXCL12 (Fig. 7A). As before, the specificities of the siRNAs were verified by Western blot analysis (Fig. 7B). These data suggest that the contribution
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**FIGURE 7.** Gαₒ and Gα₁₁ contribute, respectively, to chemotaxis of effector/memory and naive CD4⁺ T cells. A, naive and memory CD4⁺ T cells purified by cell sorting from peripheral blood were transfected with the indicated SmartPool siRNAs and harvested after 72 h. Chemotaxis assays were performed as described in the legend for Fig. 6. Data shown are the mean percentage ± S.E. of starting cells that migrated to either medium alone or CXCL12 (100 ng/ml) and are combined from three experiments. *, p < 0.05 when compared with control (Ctrl) siRNA-transfected naive cells; **, p < 0.01 when compared with control siRNA-transfected memory cells. B, lysates (15 μg) of siRNA-transfected naive and memory CD4⁺ T cells were analyzed by Western blotting (WB) for the indicated G protein α-subunits and for actin as a loading control. Data shown are from one of two experiments. For each blot, bands were analyzed by densitometry and the quantitative data, normalized to actin and then to the matching Ctrl, which was set to 100, are shown below each lane having a detectable band. C, naive and memory CD4⁺ T cells were transfected, and analyzed in chemotaxis assays as in A. Data shown are the mean percentage ± S.E. of starting cells that migrated to either medium alone or CXCL12 (100 ng/ml) from duplicate wells of one experiment and are representative of three experiments: ***, p < 0.001 when compared with the CXCL12-mediated migration of the matching control siRNA-transfected naive or memory cells. D, cord blood naive CD4⁺ T cells were cultured under TH17-polarizing conditions after which CCR6-expressing effector/memory cells were purified with magnetic beads. The purified cells were transfected with the indicated siRNAs and, after 72 h, analyzed in chemotaxis assays as described in A using the CCR6 ligand, CCL20. Data shown are the mean percentage ± S.E. of starting cells that migrated to either medium alone or CCL20 (100 ng/ml) from experiments with three donors. ***, p < 0.001 when compared with the CCL20-mediated migration of the control siRNA-transfected cells.

of Gαₒ to chemokine-dependent migration of human memory CD4⁺ T cells is equivalent to that of the abundant Gα₁₁.

In separate experiments to test the effect of knockdown of Gαₒ on primary human memory CD4⁺ T cells, we used siRNA specific for Gα₁₁, as a negative control because Gα₁₁ had not been previously detected in lymphocytes (26, 27). We were therefore surprised to find that knockdown of Gαₒ in purified naive CD4⁺ T cells led to a ~35% decrease in CXCL12-dependent chemotaxis compared with that of control naive cells, whereas knockdown of Gα₁₁ in memory CD4⁺ T cells had no effect (Fig. 7C). In agreement with our previous data, siRNA-mediated knockdown of Gαₒ inhibited CXCL12-dependent chemotaxis of memory, but not naive, CD4⁺ T cells (Fig. 7C). Because we could not identify a Gαₒ band on Western blots of naive CD4⁺ T cells using commercial antibodies (data not shown), we verified the specificity of siRNA-mediated knockdown of the appropriate G protein α-subunits by real-time RT-PCR (data not shown). Together, these data suggest that, analogous to the role of Gαₒ in memory CD4⁺ T cell subsets, the minor G protein α-subunit Gα₁₁ makes a substantial contribution to chemotactic responses in naive CD4⁺ T cells.

To extend our findings for Gαₒ in primary cells, we cultured cord blood naive CD4⁺ T cells under T17-polarizing conditions and then used magnetic beads to purify cells that expressed CCR6, which is expressed on all T17 cells (39). Purified cells were transfected with control or Gαₒ-specific siRNAs and subjected to chemotaxis assays with the CCR6 ligand, CCL20. Knockdown of Gαₒ resulted in a ~60% reduction in migration (Fig. 7D), indicating that in addition to coupling to CXCR4, Gαₒ-containing G proteins also couple to CCR6 to mediate chemotaxis in primary CD4⁺ T cells.

**Comparative Analysis of the Expression of G Protein α-Subunit mRNAs Reveals the Enrichment of Gαₒ and the Decline of Gα₁₁ in Memory T Cell Subsets**—To make direct comparisons among the different G protein α-subunit mRNAs by RT-PCR, we first established that the corresponding sets of primers and probes showed equal efficiencies of amplification from plasmids containing each of the Gα cDNA sequences (data not shown). We then used these sets of primers and probes to analyze samples from sorted populations of T cells. Our data are summarized in Fig. 8. Gα₁₁ mRNA was the most abundant G protein α-subunit mRNA in all subsets analyzed. Gαₒ mRNA abundance in CD4⁺ effector/memory cells was ~2% of that of Gα₁₁ mRNA. As mentioned previously, the abundance of Gαₒ mRNA was generally higher in naive CD8⁺ T cells than in their CD4⁺ T cell counterparts, and similar (or sometimes higher) -fold increases in the abundance of Gαₒ mRNA were observed during CD8⁺ T cell differentiation compared with that of CD4⁺ T cells. These findings are reflected in Gαₒ mRNA being found at a higher percentage of that of Gα₁₁ mRNA in CD8⁺ T cells as compared with CD4⁺ T cells (Fig. 8, C and D). Of interest, Gα₁₁ mRNA was not detectable in most of the subsets analyzed but was expressed at low abundance in naive subsets of CD4⁺ and CD8⁺ T cells (Fig. 8, A and B), which is consistent with the effect of Gα₁₁-specific siRNA on the migration of naive, but not memory, CD4⁺ T cells (Fig. 7C). The amount of Gα₁₁ mRNA was substantially lower in the more differentiated cells, sometimes below the limit of...
detection, especially in CD8⁺ T cells. Despite our inability to detect Gαi1 in Western blots of T cell protein, these findings raise the interesting possibility of a reciprocal relationship between Gαo and Gαi1 mRNA expression in human T cell subsets.

**DISCUSSION**

As far as we are aware, ours is the first description of the expression of Gαo in hematopoietic cells and the first description of differences in the content of Gα subunits in memory cells as compared with naive T cells. We have shown that among the major subsets of leukocytes in human peripheral blood, expression of Gαo was limited to T cells, and that, in contradistinction to other Gα subunits, expression of Gαo was correlated with increasingly differentiated phenotypes, with the most dramatic increment occurring in the naive to T_CM transition. Our mRNA data and knockdown experiments also suggest that expression of Gαi1 shows a reciprocal profile. Beyond the immune system, we believe that these are the first examples reported of changes in the repertoire of Gαi/o subunits as part of pathways of cellular differentiation in the adult.

Gαo is highly abundant in the central nervous system, so much so that it constitutes ~0.5–1.0% of the plasma membrane protein of the brain (41, 42). Its expression has been reported to be restricted to the central nervous system, the endocrine system, and the heart, and Gαo cannot be detected in mouse spleen or thymus (29, 43). Two main forms of Gαo are expressed, GαoA and GαoB, resulting from alternative splicing of mRNA, which produces differences in the C-terminal third of each protein (29, 31, 32, 44). In addition, there are two further isoforms of Gαo, GαoC and GαoD, which are...
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derived from posttranslational modifications of $G_{\alpha \omega A}$ and $G_{\alpha \omega B}$, respectively (45).

Within the nervous system, $G_{\alpha \omega}$ activates neuronal Ca$^{2+}$ and K$^+$ channels (46–50). Mice defective in $G_{\alpha \omega}$ show abnormalities that include tremors, impaired olfactory behavior, and decreased survival of neurons in the basal vmartrinal organ, as well as defective ion channel regulation in the heart (43, 51–53). In contrast to $G_{\alpha \omega}$, $G_{\alpha \omega i}$ is more widely distributed (54), although it also has not been detected previously in mouse lymphocytes (26) or, to our knowledge, in human lymphocytes. Apparently, mice deficient in $G_{\alpha \omega i}$ exhibit no abnormalities (55). $G_{\alpha \omega i2}$ and $G_{\alpha \omega i3}$ are considered the major $G_{\alpha \omega}$ subunits in hematopoietic and immune cells given their expression in mouse T cells (56) and the immunological, proinflammatory phenotype of $G_{\alpha \omega i2}$-deficient mice (57, 58).

We found that by contrast to brain (and other tissues (29)), human T cells contained more $G_{\alpha \omega B}$ than $G_{\alpha \omega A}$ mRNA; these are the first cells, to our knowledge, described as showing this pattern of differential splicing. We recapitulated differentiation-associated induction of $G_{\alpha \omega}$ in T cells by activating naive cord blood cells *in vitro* through the T cell receptor (TCR), and have shown that, just as for many aspects of T cell differentiation, induction of $G_{\alpha \omega}$ depended both on activation and on the cytokine environment. Of particular interest, we found preferential expression of $G_{\alpha \omega}$ under conditions inducing $T_{\text{H}17}$ cells, which have recently been recognized as a separate lineage of effector/memory CD4$^+$ T cells important for autoimmune disease (34). We have provided the first evidence that $G_{\alpha \omega}$-containing G proteins can couple to chemokine receptors that are expressed on T cells, including the $T_{\text{H}17}$-associated receptor, CCR6 (37–39). Finally, we have shown that siRNA-mediated depletion of $G_{\alpha \omega}$ in primary naive CD4$^+$ T cells and of $G_{\alpha \omega}$ in memory CD4$^+$ T cell subsets inhibited CXCR4-mediated migration to a similar extent to knocking down the major $G_{\omega}$ subunit, $G_{\alpha \omega i2}$. These data suggest that CXCR4, and perhaps other GPCRs, switch their Go subunit usage from $G_{\alpha \omega i2}$ in naive CD4$^+$ T cells to $G_{\alpha \omega}$ in memory CD4$^+$ T cell subsets and that the contribution of these Go subunits to GPCR-mediated functions belies their relatively low abundance in these cells. The discordance between functional importance and relative abundance was also reported for $G_{\alpha \omega i2}$ as compared with $G_{\alpha \omega i1}$ in HeLa cells (23). It is notable that in those experiments, knocking down some species of $G_{\alpha \omega i0}$ subunits resulted in “compensatory” increases in others (23). We did not observe such changes among the limited number of Go proteins that we analyzed, perhaps because we harvested cells at only 3 days after transfection with siRNA and not the 5 days used in the previous study and/or because of cell type-specific differences.

We presume that $G_{\alpha \omega}$ and $G_{\alpha \omega i}$ have roles in T cells beyond chemokine receptor signaling. One possible role for $G_{\alpha \omega}$ would be in signaling from GPCRs such as species of opioid, muscarinic acetylcholine (mACHR), and somatostatin receptors, which are expressed in the central nervous system, have been shown to use $G_{\alpha \omega}$ (59–61), and can be expressed by T cells (62–69).

The published data on downstream targets of activated $G_{\alpha \omega}$ raise some additional, interesting possibilities as to specific roles for $G_{\alpha \omega}$ in memory T cells. For example, channels with characteristics of L-type Ca$^{2+}$ channels are found in T cells, and inhibitor studies have shown that these channels play a role in mediating TCR-induced lymphocyte activation and proliferation both *in vitro* and *in vivo* (70, 71). Given the documented, specific role for $G_{\alpha \omega}$ in regulating these channels in the heart (43), it is possible that $G_{\alpha \omega}$ contributes to the differences in Ca$^{2+}$ signals, and other consequences of TCR activation that distinguish memory from naive T cells (72). Connections between chemokine receptors, G proteins, and the TCR are suggested by reports that CXCL12 acts as a costimulator for the activation of CD4$^+$ T cells (73) and that CXCR4 activates $\zeta$ chain-associated protein kinase of 70 kDa (Zap70) (74) and associates directly with the TCR (75). In addition, $G_{\alpha \omega i2}$ mediates inhibition of TCR-induced Ca$^{2+}$ signals and cytokine production (27).

Finally, $G_{\alpha \omega}$ is thought to mediate the activities of Wnt receptors (frizzled proteins) in mouse cells (76) and in Drosophila (77, 78), and the effectors of the Wnt pathway, $\beta$-catenin and lymphoid-enhancing factor/T cell factor proteins, are important, respectively, for thymocyte survival (79) and T cell differentiation (80). A possible role for (G$_{\alpha \omega i2}$-mediated) Wnt signaling in the survival, proliferation, or both of memory T cells is intriguing but as yet unexplored. However, the accumulating data that chemokine receptors are involved in stimulating cell growth and proliferation (81, 82), and that GPCRs other than frizzled proteins can activate components of the Wnt pathway (83) raise the possibility that chemokine receptor signaling through $G_{\alpha \omega}$ to the $\beta$-catenin pathway in memory T cells could contribute to their proliferation. However, given the lack of $G_{\alpha \omega}$ protein in mouse lymphocytes, any of the possible roles that we have suggested for $G_{\alpha \omega}$ would by necessity be specific to human T cells, representing a clear difference between the immune system in humans and mice.

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**REFERENCES**

1. Seder, R. A., and Ahmed, R. (2003) *Nat. Immunol.* **4**, 835–842
2. Berke, G. (1995) *Immunol. Today* **16**, 343–346
3. Harty, J. T., Tvinnereim, A. R., and White, D. W. (2000) *Annu. Rev. Immunol.* **18**, 275–308
4. Sallusto, F., Geginat, J., and Lanzavecchia, A. (2004) *Annu. Rev. Immunol.* **22**, 745–763
5. Rabin, R. L., Park, M. K., Liao, F., Swofford, R., Stephany, D., and Farber, J. M. (1999) *J. Immunol.* **162**, 3840–3850
6. Hadcock, J. R., Ros, M., Watkins, D. C., and Malbon, C. C. (1990) *J. Biol. Chem.* **265**, 14784–14790
7. Loganzo, F. Jr., and Fletcher, P. W. (1992) *Mol. Endocrinol.* **6**, 1259–1267
8. Loganzo, F. Jr., and Fletcher, P. W. (1993) *Mol. Endocrinol.* **7**, 434–440
9. Bahouth, S. W. (1995) *Biochem. J.* **307**, 831–841
10. El Jamali, A., Rachdaoui, N., Dib, K., and Corrêa, C. (1998) *J. Neurochem.* **71**, 2271–2277
78. Katanaev, V. L., and Tomlinson, A. (2006) Proc. Natl. Acad. Sci. U.S.A. 103, 6524–6529
79. Xie, H., Huang, Z., Sadim, M. S., and Sun, Z. (2005) J. Immunol. 175, 7981–7988
80. Timm, A., and Grosschedl, R. (2005) Curr. Top. Microbiol. Immunol. 290, 225–252
81. Sutton, A., Friand, V., Brulé-Donneger, S., Chaigneau, T., Ziol, M., Sainte-Catherine, O., Poiré, A., Saffar, L., Kraemer, M., Vassy, J., Nahon, P., Salzmann, J. L., Gattegno, L., and Charnaux, N. (2007) Mol. Cancer Res. 5, 21–33
82. Cho, H. H., Kyoung, K. M., Seo, M. I., Kim, Y. J., Bae, Y. C., and Jung, J. S. (2006) Stem Cells Dev. 15, 853–864
83. Yang, M., Zhong, W. W., Srivastava, N., Slavin, A., Yang, J., Hoey, T., and An, S. (2005) Proc. Natl. Acad. Sci. U.S.A. 102, 6027–6032