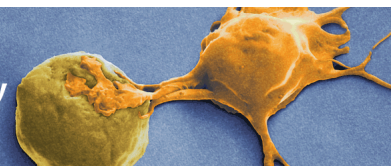




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This information is current as of May 21, 2012.

J Immunol published online 16 May 2012

<http://www.jimmunol.org/content/early/2012/05/16/jimmunol.1103781>

Supplementary Material <http://www.jimmunol.org/content/suppl/2012/05/16/jimmunol.1103781.DC1.html>

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RGS16 Attenuates Pulmonary Th2/Th17 Inflammatory Responses

Sucharita P. Shankar,* Mark S. Wilson,[†] Jeffrey A. DiVietro,* Margaret M. Mentink-Kane,[†] Zhihui Xie,* Thomas A. Wynn,[†] and Kirk M. Druey*

The regulators of G protein signaling (RGS) protein superfamily negatively controls G protein-coupled receptor signal transduction pathways. RGS16 is enriched in activated/effector T lymphocytes. In this paper, we show that RGS16 constrains pulmonary inflammation by regulating chemokine-induced T cell trafficking in response to challenge with *Schistosoma mansoni*. Naive *Rgs16*^{-/-} mice were “primed” for inflammation by accumulation of CCR10⁺ T cells in the lung. Upon pathogen exposure, these mice developed more robust granulomatous lung fibrosis than wild-type counterparts. Distinct Th2 or putative Th17 subsets expressing CCR4 or CCR10 accumulated more rapidly in *Rgs16*^{-/-} lungs following challenge and produced proinflammatory cytokines IL-13 and IL-17B. CCR4⁺*Rgs16*^{-/-} Th2 cells migrated excessively to CCL17 and localized aberrantly in challenged lungs. T lymphocytes were partially excluded from lung granulomas in *Rgs16*^{-/-} mice, instead forming peribronchial/perivascular aggregates. Thus, RGS16-mediated confinement of T cells to Schistosome granulomas mitigates widespread cytokine-mediated pulmonary inflammation. *The Journal of Immunology*, 2012, 188: 000–000.

Chemokines dictate coordinated movement of leukocytes through lymphoid organs and sites of inflammation. Naive and activated leukocyte populations express unique chemokine receptors, and gene-targeting studies have implicated specific chemokines and receptors in leukocyte activation, differentiation, and lymphoid organ development (1). Schistosomiasis, induced by infection with the helminth *Schistosoma mansoni* (among other species), represents a global disease burden because of resultant hepatic fibrosis and portal hypertension (2). *S. mansoni* cercariae breach host skin and develop into adult male–female worm pairs that generate hundreds of eggs per day (3, 4). Eggs then enter circulation or embolize in host tissues such as liver, intestine, and lung, where a granulomatous reaction and fibrosis develop in an effort to sequester the foreign eggs (3). In published mouse models of schistosomiasis, the pulmonary granulomatous response is initiated by CD4⁺ Th2 cells and their secreted cytokines, particularly IL-13 (5). Although chemokine factors mediating Th2 recruitment to lungs acutely challenged with *S. mansoni* eggs have been suggested (e.g., CCL17/CCL22 and CCR4/8), signaling pathways involved in pulmonary inflammation have not been fully defined (6).

Chemokine receptors are G protein-coupled receptors (GPCRs) linked to Gαi and possibly Gαq to induce chemotaxis (7). The primary signal transducer of GPCRs, the heterotrimeric G protein complex of α, β, and γ subunits, induces pathway activation through GDP–GTP exchange on Gα and stimulation of numerous effectors including kinases, phospholipases, and ion channels (8, 9). The intrinsic GTPase activity of the α subunit, which promotes Gα reassociation with βγ to form an inactive heterotrimer, terminates ligand-induced signaling. The regulators of G protein signaling (RGS) superfamily, which has >30 members in mammalian cells, negatively regulates G protein activity (10). All RGS proteins contain a characteristic 120-aa “RGS box,” which facilitates binding to Gα subunits and GTPase-accelerating protein (GAP) activity (11). RGS GAP activity hastens GPCR pathway inactivation by catalyzing the GTPase reaction. Although molecular determinants of RGS activity have been elucidated over the past decade, most physiological functions of RGS proteins in mammals remain unknown. Gαi inactivation by pertussis toxin disrupts physiological hematopoietic cell trafficking including thymic emigration, transendothelial leukocyte migration into lymph nodes (LNs), and Ag-induced recruitment of cells to inflamed tissue (7). Because RGS proteins are physiologically relevant inhibitors of Gαi, they are poised to regulate chemokine-mediated responses in vivo (7).

RGS16 was initially discovered as an IL-2–dependent activation gene in human T lymphocytes (12). *RGS16* may control Th2 lymphocyte migration in vivo because it is upregulated in activated human Th1 and Th2 cells relative to naive T cells, and *RGS16* overexpression inhibits Th lymphocyte chemotaxis in vitro (13). To explore intracellular regulation of chemokine pathways in pulmonary inflammation, we generated *Rgs16*^{-/-} mice and studied their response to sensitization with *S. mansoni* egg Ag followed by an i.v. bolus of live *S. mansoni* eggs (14). These studies revealed that *RGS16* inhibits Th2 chemotaxis to chemokines including CCL17, which constrains T cell localization to Schistosome egg granulomas, thereby reducing the tissue-damaging effects of Th2-induced pulmonary inflammation by confining cytokines to specific regions(s) of the lung.

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Received for publication December 23, 2011. Accepted for publication April 16, 2012.

This work was supported by the Intramural Research Program of the National Institute of Allergy and Infectious Diseases, National Institutes of Health (Grant AI000746; to K.M.D.).

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The online version of this article contains supplemental material.

Abbreviations used in this article: C_T, cycle threshold; GAP, GTPase-accelerating protein; GPCR, G protein-coupled receptor; LN, lymph node; MLN, mediastinal lymph node; qPCR, quantitative PCR; RGS, regulator of G protein signaling; SEA, Schistosome egg Ag; Socs2, suppressor of cytokine signaling 2; WT, wild-type.

Materials and Methods

Generation of *Rgs16*^{-/-} mice

C57BL/6 wild-type (WT) mice were purchased from The Jackson Laboratory. *Rgs16*^{-/-} mice were generated directly onto a C57BL/6 background as outlined in Fig. 1. All mice were housed in pathogen-free conditions and research performed in accordance with protocols (LAD3e and LPD16e) approved by the National Institutes of Health Institutional Animal Care and Use Committee. Male or female mice between 6 and 12 wk of age were used for all experiments.

S. mansoni lung challenge model

Mice were sensitized i.p. with 5000 *S. mansoni* eggs that were derived previously from sterile, LPS-free BALB/c mice harboring a liver infection (3). Fourteen days later, mice were challenged i.v. with 5000 live eggs of a Puerto Rican strain of *S. mansoni* (NMRI) as described elsewhere (3). The challenged mice were euthanized at 4 or 7 d post i.v. injection by CO₂ inhalation. Lungs, spleens, and mediastinal LNs (MLNs) were harvested from both groups of challenged mice for analysis.

Histopathology and immunohistochemistry

Organs were fixed in 10% neutral-buffered formalin (EMD Chemicals) and embedded in paraffin. Tissue sections (5 μ m) were evaluated for granuloma volume and diameter by H&E staining, and lung fibrosis was scored, based on Picrosirius red stain for collagen using published scoring methods (15). Giemsa stains were used to quantify eosinophil numbers. The individual who scored all histological features was blinded to the experimental design. Lungs harvested at 7 d post-i.v. challenge were stained for the following markers: anti-CD3 (1:100) (DakoCytomation) or anti-CCR10 (1:500) (Abcam). A polyclonal anti-RGS16 Ab (Dru-4) was generated by immunizing rabbits with an N-terminal RGS16 peptide (MCRTLATFPNTC-amide) and collection of serum. The Ab was affinity purified using a peptide-conjugated resin (Spring Valley Laboratories). Slides were deparaffinized and washed twice in distilled water for 5 min at room temperature. After pretreatment for Ag retrieval (20–40 min, 75–90°C), the slides were washed again, and endogenous peroxidase activity was inhibited with H₂O₂ for 10 min at room temperature. Nonspecific binding was blocked using 5% BSA (Sigma-Aldrich) in PBS for 20 min at room temperature. Sections were incubated with primary Ab or matched isotype controls (1:500) overnight at room temperature, washed, and incubated with the biotinylated secondary Ab (1:500, 30 min at room temperature) and finally incubated with streptavidin-HRP (1:400, 30 min at room temperature) (Alpha Diagnostics). The signal was detected with 3,3'-diaminobenzidine (1–20 min at room temperature). Sections were counterstained in Carazzi hematoxylin (HistoServ) for 2 min at room temperature, followed by dehydration in graded alcohols (75, 95, and 100%) for 1 min each at room temperature and xylene (Sigma-Aldrich). Images were obtained with a Leica DMI 4000B microscope and quantified using Image Pro Plus software (Media Cybernetics).

Real-time PCR and gene expression arrays

Total RNA was isolated using the RNeasy mini kit (Qiagen), followed by DNase I treatment (Invitrogen). cDNA was synthesized from 0.25 to 1 μ g RNA using the Superscript III First-Strand Synthesis kit (Invitrogen). Real-time RT-quantitative PCR (qPCR) was performed using the TaqMan strategy (Applied Biosystems). No reverse transcriptase controls were included to verify DNase digestion. Gene expression (*Rgs16* [NM_011267.3], suppressor of cytokine signaling 2 [*Socs2*] [NM_007706.3], *Ccr10* [NM_007721.4], *Ccl28* [NM_020279.3], *Il13* [NM_016971.2], *Il17b* [NM_019508.1], or the internal reference control β -actin [NM_007393.3]) was measured by multiplex PCR using probes labeled with FAM. The simultaneous measurement of target gene-FAM and β -actin-FAM allowed for normalization of the amount of cDNA added per sample. Duplicate PCRs were performed using the TaqMan Universal PCR Master Mix and the ABI 7500 Standard or Step One Plus sequence detection systems (Applied Biosystems) according to the following thermal cycle routine: 95°C for 10 min, followed by 40 cycles of 95°C for 1 min and 60°C for 20 min. A comparative cycle threshold (C_T) method was used to determine gene expression relative to the no-sample control (calibrator). Steady-state mRNA levels were expressed as an n -fold difference relative to the calibrator. For each sample, the *RGS16* C_T value was normalized with the formula: $\Delta C_T = C_{T \text{ RGS16}} - C_{T \beta\text{-ACTIN}}$. To determine relative expression levels, the following formula was used: $\Delta\Delta C_T = \Delta C_{T \text{ sample}} - \Delta C_{T \text{ calibrator}}$. Relative expression is presented as $2^{-\Delta\Delta C_T}$. Multiplex qPCR was performed using a mouse chemokines and receptors qPCR array (SA Biosciences), according to the manufacturer's instructions.

Cytokine analysis

Single-cell suspensions were prepared from MLNs harvested 7 d following i.v. challenge; 0.5×10^6 cells/well were plated in triplicate wells of 96-well round polystyrene plates (Corning) in RPMI 1640 medium plus 10% FBS (Invitrogen). Cells were left untreated or stimulated with Schistosoma egg Ag (SEA) from *S. mansoni* in PBS (10 μ g/ml final concentration) for 3 d at 37°C. Cell supernatants were collected, and cytokine levels were measured with a fluorescence-based Bio-Plex Pro mouse cytokine Th1/Th2 assay (Bio-Rad), according to the manufacturer's protocols. Cells were fixed by the addition of PBS containing 2% BSA and 4% paraformaldehyde (Electron Microscopy Sciences). ELISAs were performed to quantify secretion of mouse cytokines IL-4, IL-13, IFN- γ , IL-17A, or IL-17B (R&D Systems) in accordance with the manufacturer's protocols.

Flow cytometry and intracellular staining

Organs were harvested 4 or 7 d postchallenge and converted to single-cell suspensions followed by fixation in PBS containing 2% BSA and 4% paraformaldehyde. Surface markers were analyzed with the following Abs: CD3 (17A2), B220 (RA3-6B2), CD11c (N418) (eBioscience), CD4 (RM4-5), CD8 (53-6.7), CCR3 (83103), CXCR4 (2B11) CXCR3 (CXCR3-173), CCR6 (C34-3448) (BD Biosciences), CCR4 (2G12) (Biolegend), or CCR10 (248918) (R&D Systems), and cytokines were detected with anti-IL-5 (TRFK5) (BD Biosciences), anti-IL-13 (ebio13A), anti-IFN- γ (XMG1.2) (eBioscience), or anti-IL-17B (R&D Systems). For intracellular cytokine staining, cells were activated with PMA (50 ng/ml) and ionomycin (0.5 μ M) (Sigma-Aldrich) for 6 h at 37°C in the presence of brefeldin A (BD Biosciences). Cells were permeabilized with PBS/0.1% saponin (Sigma-Aldrich) and blocked with 5% PBS/milk (Santa Cruz Biotechnology) (15 min, 4°C) and Fc block (BD Biosciences). Fixed permeabilized cells were stained in the dark with fluorescently conjugated Abs (1 h, 4°C) and washed twice with FACS buffer. Samples were analyzed by flow cytometry using an LSR Fortessa (BD Biosciences), and data were analyzed using FlowJo (Tree Star).

In vitro Th1, Th2, or Th17 culture

Single-cell suspensions were generated from peripheral LNs from naive mice. Naive T cells (CD4⁺CD62L⁺) were isolated using the naive T cell isolation kit II (Miltenyi Biotec). A total of 3×10^5 cells/well were plated in 6-well plates precoated with anti-CD3 (1 μ g/ml) and anti-CD28 (3 μ g/ml) containing RPMI 1640 medium plus 10% FBS, recombinant mouse IL-4 (10 ng/ml) (PeproTech), anti-mouse IFN- γ (10 μ g/ml) (BD Biosciences), and 50 μ M 2-ME for 3 d at 37°C. For differentiation into a Th1 phenotype, naive T cells were cultured in media containing IL-2 (10 ng/ml), IL-12 (10 ng/ml) (R&D Systems), and anti-IL-4 (10 μ g/ml) (eBioscience), referred to as the Th1 mixture. For differentiation into a Th17 phenotype, naive T cells were cultured in media containing IL-23 (5 ng/ml), IL-21 (100 ng/ml), IL-6 (10 ng/ml), human TGF- β (5 ng/ml), anti-IL-4 (10 μ g/ml), anti-IFN- γ (10 μ g/ml) (R&D Systems), and anti-IL-12 (10 μ g/ml) (BD Biosciences), referred to as the Th17 mixture. The differentiated activated Th1 or Th2 cells were then expanded in medium containing recombinant mouse IL-2 (10 ng/ml) and IL-7 (5 ng/ml) (PeproTech) or in IL-2 alone for Th17 cells.

Generation and purification of TAT fusion proteins

The coding region of human *RGS16* was amplified by PCR using pcDNA3.1-RGS16 as a template and subcloned in-frame into the plasmid vector pTat-H₆HA-GFP, which results in an N-terminally tagged GFP-RGS16 (16). Recombinant TAT fusion proteins were expressed in *Escherichia coli* and purified by nickel-affinity chromatography as described previously (17). TAT proteins (TAT-GFP-RGS16 or TAT-GFP control) were added directly to T cell cultures for 2 h prior to stimulus addition.

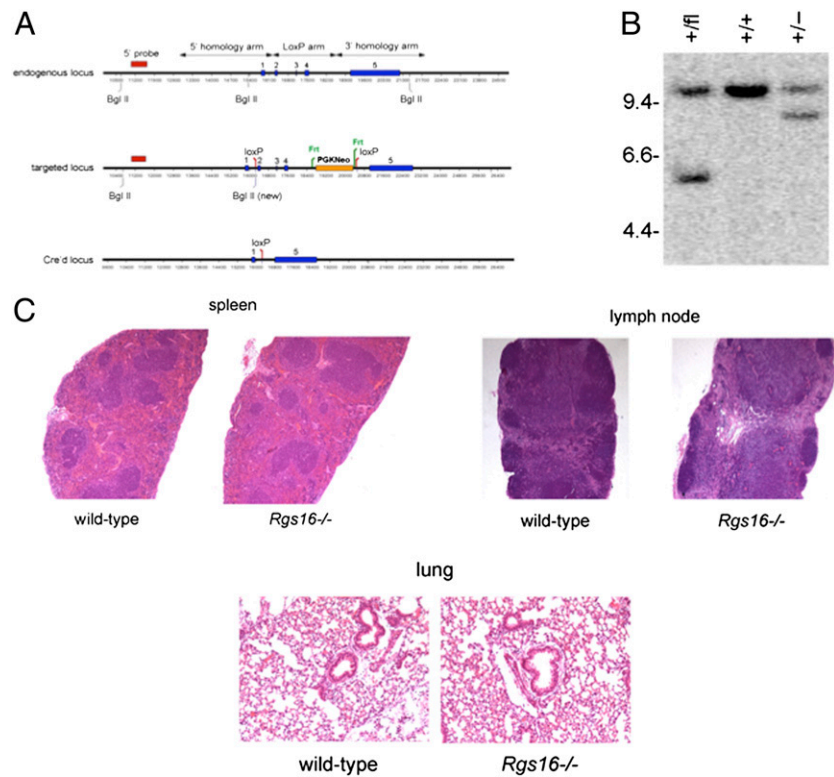
Chemotaxis assay

Differentiated Th2 cells (0.5×10^6 cells/well) were plated in upper wells of 5- μ m-pore polycarbonate membrane Transwell migration chambers containing RPMI 1640 medium plus 0.5% BSA (Corning). The bottom wells contained chemokines (CXCL12, CCL21, CCL17, CXCL9, or CCL20) (R&D Systems). Control wells, in which upper and lower chambers had equivalent chemokine concentrations, were used to determine chemokinesis. Cells migrated to the lower chamber were counted after 3 h by flow cytometry using a FACSCalibur (BD Biosciences).

CFSE assay for analysis of cell proliferation

Cells were labeled using the CellTrace CFSE cell proliferation kit (Invitrogen) plated in 96-well plates left untreated or precoated with anti-CD3/

FIGURE 1. Generation and characterization of *Rgs16*^{−/−} mice. **(A)** A targeting vector was generated by flanking a floxed-Neo^r cassette (PGKNeo) by two homologous arms of the endogenous *Rgs16* gene. Shown are restriction maps and exons (solid vertical bars) of the endogenous and targeted *Rgs16* loci (blue bars). **(B)** WT and floxed alleles were identified by Southern blotting of BglII-digested genomic DNA with a 5′ probe (red), yielding 10.6- or 5.5-kb fragments, respectively (middle). Mice homozygous for the floxed allele were crossed with Rosa-Cre strain, resulting in deletion of PGKNeo and exons 2–4, which was identified as an 8.3-kb fragment. **(C)** Architecture of spleens, lungs, and peripheral LNs in naive WT or *Rgs16*^{−/−} mice was evaluated by H&E staining. Original magnification ×5.



anti-CD28. Cells were harvested 4 d later, and CFSE dilution profiles were evaluated by flow cytometry.

Statistical analysis

Data sets were compared by Student *t* test for two groups or one-way ANOVA for multiple groups using Graph Pad Prism software. Differences with *p* ≤ 0.05 were considered significant.

Results

Generation of *Rgs16*^{−/−} mice

Rgs16 was targeted by flanking exons 2–4 with *loxP* sites (Fig. 1A). We generated knockouts by crossing mice with floxed *Rgs16* allele with Rosa-Cre mice, which have the gene encoding Cre recombinase inserted into the ubiquitous *Rosa26* locus. Correct targeting of the *Rgs16* allele was confirmed by Southern blot analysis (Fig. 1B). Homozygous mice were viable and fertile and exhibited no gross phenotypic abnormalities. Architecture of the spleens, lungs, and peripheral LNs in naive *Rgs16*^{−/−} mice was comparable to WT mice by light microscopy (Fig. 1C). Percentages of CD4⁺ and CD8⁺ T cells, B cells, and dendritic cells in the spleen, lungs, and LNs were similar in WT and *Rgs16*^{−/−} mice (Table I).

RGS16 inhibits Th1 and Th2 chemotaxis

To investigate function(s) of RGS16 in T cell-dependent inflammation, we examined its expression pattern in mouse effector

T cells and their chemotactic responses. Compared with naive (CD4⁺CD62L⁺) T cells isolated from peripheral LNs, activated T cells were polarized to a Th2 phenotype by IL-4, and anti-IFN-γ Ab expressed 5-fold more *Rgs16* mRNA, whereas those polarized to Th1 phenotype by a Th1 mixture or Th17 phenotype by a Th17 mixture expressed 2-fold more *Rgs16* mRNA (Fig. 2A). We measured chemotaxis of naive CD4 T cells to gradients of CCL21 and CXCL12 because these chemokines have an important function in homeostatic lymphocyte trafficking in lymphoid organs (18). Consistent with relatively low *Rgs16* expression in naive CD4 T cells, CXCL12 and CCL21 induced equivalent chemotaxis of naive splenocytes or naive splenic CD4 T cells from unchallenged WT and *Rgs16*^{−/−} mice (Fig. 2B). Th2 cells up-regulate several “inflammatory” chemokine receptors not typically expressed by naive cells including CCR4 and CCR8, which mediate trafficking to inflamed tissues containing CCL17 (19). Accordingly, in vitro-differentiated Th2 cells from mice lacking RGS16 migrated much more toward CCL17 gradients than WT counterparts (Fig. 2C). At the CCL17 concentration inducing a peak response (50 nM), nearly double the number of RGS16-deficient Th2 cells than WT cells migrated to the lower chamber (~80 versus 40%).

In contrast to Th2 cells, Th1 and Th17 cells express a distinct set of chemokine receptors including CXCR3 and CCR5 (Th1) or

Table I. Cellular composition of spleens, LNs, or lungs of naive WT or *Rgs16*^{−/−} mice

Tissue	Mice	CD3 ⁺ (%)	CD3 ⁺ CD4 ⁺ (%)	CD3 ⁺ CD8 ⁺ (%)	B220 ⁺ (%)	CD11c ⁺ (%)
Spleen	WT	41.32 ± 15.42	59.13 ± 5.6	5.06 ± 6.33	49.04 ± 5.51	8.24 ± 8.17
Spleen	<i>Rgs16</i> ^{−/−}	48.69 ± 5.47	58.39 ± 1.55	6.31 ± 6.56	47.8 ± 6.59	4.90 ± 4.05
Lymph node	WT	60.27 ± 6.32	55.52 ± 4.84	N.D.	30.53 ± 4.54	N.D.
Lymph node	<i>Rgs16</i> ^{−/−}	63.23 ± 4.27	54.06 ± 1.4	N.D.	32.08 ± 4.62	N.D.
Lung	WT	18.14 ± 3.81	24.12 ± 10.99	0.105 ± 0.06	6.99 ± 2.10	6.54 ± 2.18
Lung	<i>Rgs16</i> ^{−/−}	23.79 ± 10.17	21.06 ± 18.24	N.D.	7.29 ± 5.85	6.92 ± 3.99

B lymphocytes, T lymphocytes, and dendritic cell percentages in lymphoid organs and lungs were determined by means of flow cytometry using the indicated markers. Results are mean ± SEM from six mice of each genotype. N.D., Not detectable.

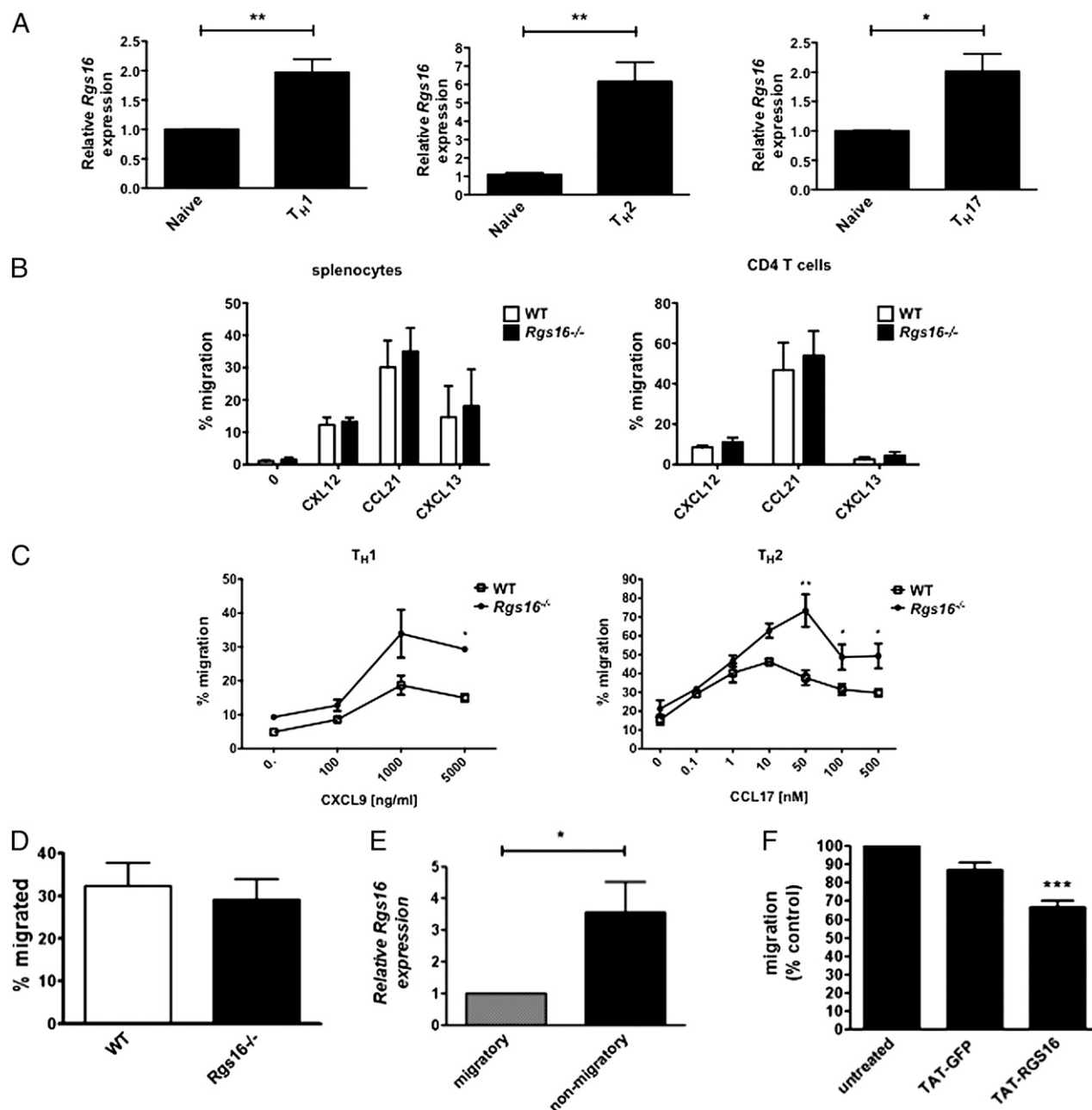


FIGURE 2. RGS16 inhibits Th2 chemotaxis. **(A)** Naive T cells were isolated from peripheral LNs and differentiated into Th1, Th2, or Th17 cells as outlined in *Materials and Methods*. Relative *Rgs16* expression was measured by real-time PCR (** $p = 0.005$, * $p < 0.01$; unpaired t test). **(B)** Whole splenocytes or splenic CD4 T cells C57BL/6 and *Rgs16*^{-/-} mice were exposed to chemokines in Transwell plates for 3 h at 37°C, followed by enumeration of migrated cells by flow cytometry. **(C)** Migration of Th1 or Th2 cells toward CXCL9 or CCL17 gradients, respectively, at the indicated concentrations was measured as in **(B)** (* $p = 0.04$, ** $p < 0.003$; unpaired t test). **(D)** Chemokinesis was measured by incubating cells with equimolar concentrations of CCL17 (50 nM) in the upper and lower chambers of Transwell plates, followed by enumeration of cells by flow cytometry. **(E)** *Rgs16* expression in cells migrated to the lower chamber of CCL17-containing Transwell plates ("migratory") was compared with cells retained in the upper chamber ("nonmigratory") by real-time PCR (* $p = 0.04$, paired t test). **(F)** Th2 cells from *Rgs16*^{-/-} mice were left untreated or preincubated with TAT-GFP or TAT-RGS16 (60–500 nM) for 1 h prior to exposure to CCL17 gradients in Transwell assays (*** $p < 0.001$; one-way ANOVA, TAT-RGS16 compared with untreated or TAT-GFP). All data are mean $\pm$ SEM of three to four independent experiments using cells from 1 mouse/group in each.

CCR6 (Th17) (20–22). Similar to the behavior of RGS16-deficient Th2 cells, Th1 cells differentiated from *Rgs16*^{-/-} mice migrated significantly more toward a gradient of CXCL9 (CXCR3 ligand) than did WT counterparts (Fig. 2C). For unclear reasons, we did not observe significant chemotaxis of murine Th17 cells toward CCL20 gradients in Transwell assays, independent of genotype (Supplemental Fig. 1). WT and *Rgs16*^{-/-} T cells migrated to a similar extent in the presence of equivalent chemokine concentrations in the upper and lower chambers, indicating that the

loss of RGS16 affected chemotaxis rather than chemokinesis (Fig. 2D). *Rgs16* expression correlated with chemokine resistance. WT T cells retained in the upper chamber in the presence of a CCL17 gradient for 3 h ("nonmigratory") expressed significantly more *Rgs16* than cells migrating to the lower chamber during that time period ("migratory") (Fig. 2E). Finally, because WT and *Rgs16*^{-/-} Th-polarized subsets expressed similar levels of surface chemokine receptors including CXCR3 for Th1 cells, CXCR4, CCR10, or CCR4 for Th2 cells, and CCR6 for Th17 cells, these

results indicated that RGS16 inhibits chemotaxis downstream of receptors (Supplemental Fig. 1). Consistent with this interpretation, reconstitution of RGS16-deficient Th2 cells with TAT-RGS16 reduced CCL17-evoked chemotaxis compared with untreated cells or cells incubated with a control TAT protein (GFP) (Fig. 2F). Collectively, these results suggest that RGS16 directs trafficking of Th1 or Th2 lymphocytes by curbing their response to chemokine.

Rgs16^{-/-} mice have increased granulomatous lung fibrosis after challenge with *S. mansoni*

To evaluate Th2 trafficking in vivo in *Rgs16*^{-/-} mice and its impact on an acute inflammatory response, we sensitized mice with *S. mansoni* eggs i.p., followed by i.v. injection of eggs 14 d later (Fig. 3A). Mice were sacrificed 4 and 7 d after the i.v. inoculation, at which time *S. mansoni* eggs embolize in the lungs, resulting in synchronous pulmonary inflammation characterized by granulomas, infiltration of neutrophils, eosinophils, and Th2 lymphocytes, and collagen deposition/fibrosis (23). Consistent with a role for RGS16 in the regulation of host responses to *S. mansoni*, *Rgs16* mRNA expression was increased in the lungs of sensitized challenged mice 7 d following the i.v. Schistosome challenge (Fig. 3B). We also detected RGS16 in the lungs of challenged mice by immunohistochemistry (Fig. 3C). RGS16⁺ cells localized predominantly in granulomas surrounding lodged *S. mansoni* eggs. The lungs of *Rgs16*^{-/-} mice developed significantly more inflammation and fibrosis than those of WT mice (Fig. 3D). Fibrosis scores (Fig. 3E), granuloma volumes (Fig. 3F), and eosinophil scores (Fig. 3G) were all higher in lungs of knockout mice compared with WT C57BL/6 mice. These results indicated that RGS16 constrains acute granulomatous pulmonary fibrosis induced by *S. mansoni* infection.

RGS16 deficiency induces anomalous lymphocyte trafficking in vivo

To determine which lymphocyte subsets mediated the atypical fibrotic response of *Rgs16*^{-/-} mice lungs to *S. mansoni*, we immunophenotyped pulmonary inflammatory infiltrates by means of flow cytometry. Although we detected no substantial differences in the percentages or total numbers of B and T cells in the lungs of challenged WT and *Rgs16*^{-/-} mice, there was a skewed Th2 response in knockout mice as evidenced by increased percentages of IL-13⁺ T cells in the lungs 4 d following helminth challenge. The ratio of IL-13⁺ (Th2)/IFN-γ⁺ (Th1) frequencies was significantly increased in *Rgs16*^{-/-} lungs relative to WT (Fig. 4A, 4B). Because Th cells from WT and *Rgs16*^{-/-} mice exhibited comparable rates of differentiation, proliferation, and survival in vitro (Supplemental Fig. 2; data not shown), aberrant cell growth and/or death probably did not account for the differences in the cellular composition observed.

Splenic CD4 T cells isolated from challenged *Rgs16*^{-/-} mice migrated more toward CCL17 gradients than cells from WT mice (Fig. 4C). Thus, irregular trafficking patterns of fibrosis-inducing IL-13⁺ Th2 cells might contribute to the enhanced inflammation in *Rgs16*^{-/-} mice following helminth challenge. Consistent with this hypothesis, we observed starkly atypical T lymphocyte localization patterns in the lungs of RGS16-deficient mice following *S. mansoni* inoculation compared with WT. Whereas T cells were distributed uniformly within fibrotic granulomas surrounding embolized eggs in WT lungs, they were restricted to the periphery of granulomas in the lungs of *Rgs16*^{-/-} mice (Fig. 5A). In addition, we detected dense perivascular/peribronchial T cell aggregates that were largely absent in WT mice (Fig. 5B, 5C). These data

FIGURE 3. Enhanced pulmonary inflammation in *Rgs16*^{-/-} mice challenged with *Schistosoma mansoni*. (A) Experiment layout. (B) *Rgs16* expression in naive or *S. mansoni*-challenged lungs was measured by real-time PCR (**p* = 0.04, unpaired *t* test). (C) RGS16 expression detected by immunohistochemistry using an RGS16 Ab in the lungs of WT or knockout mice as indicated. Images are representative of three to five mice per group. Original magnification ×5. (D) Picrosirius red staining for collagen in the lungs of WT or *Rgs16*^{-/-} mice 7 d following inoculation with *S. mansoni* eggs. Original magnification ×5 or ×40. (E–G) Fibrosis scores (E), granuloma volumes (F), and eosinophil scores (G) were evaluated in WT and *Rgs16*^{-/-} lungs 7 d after helminth challenge. Data represent eight mice per group evaluated in two independent experiments (**p* < 0.04; unpaired *t* test).

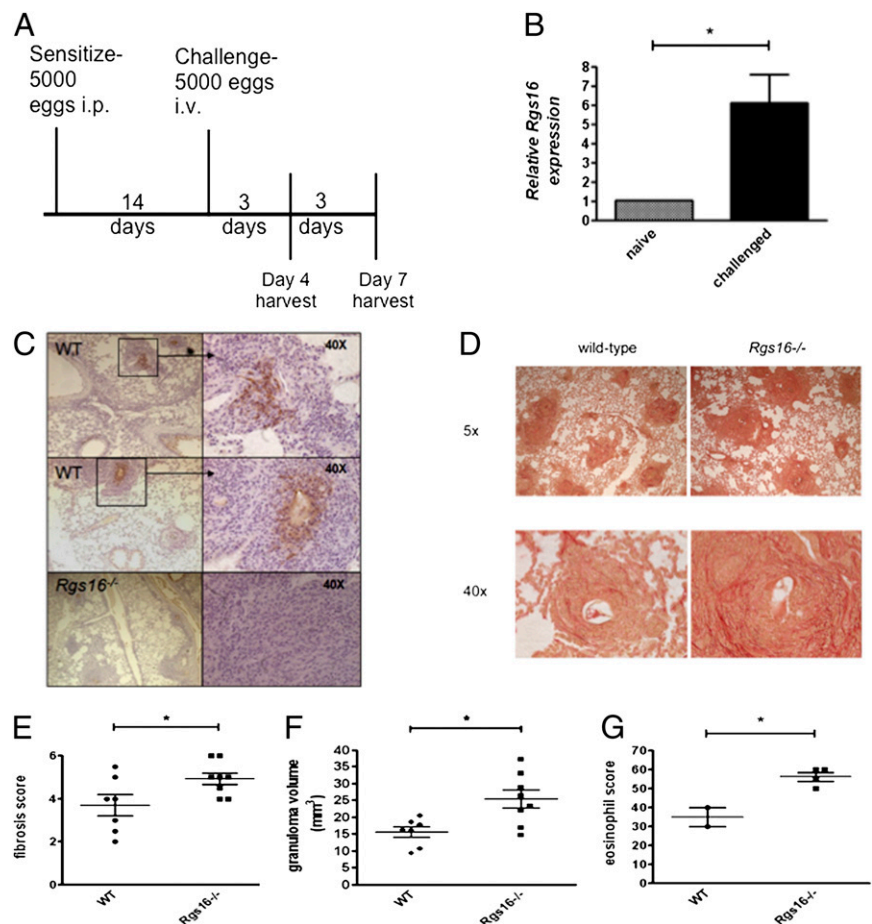
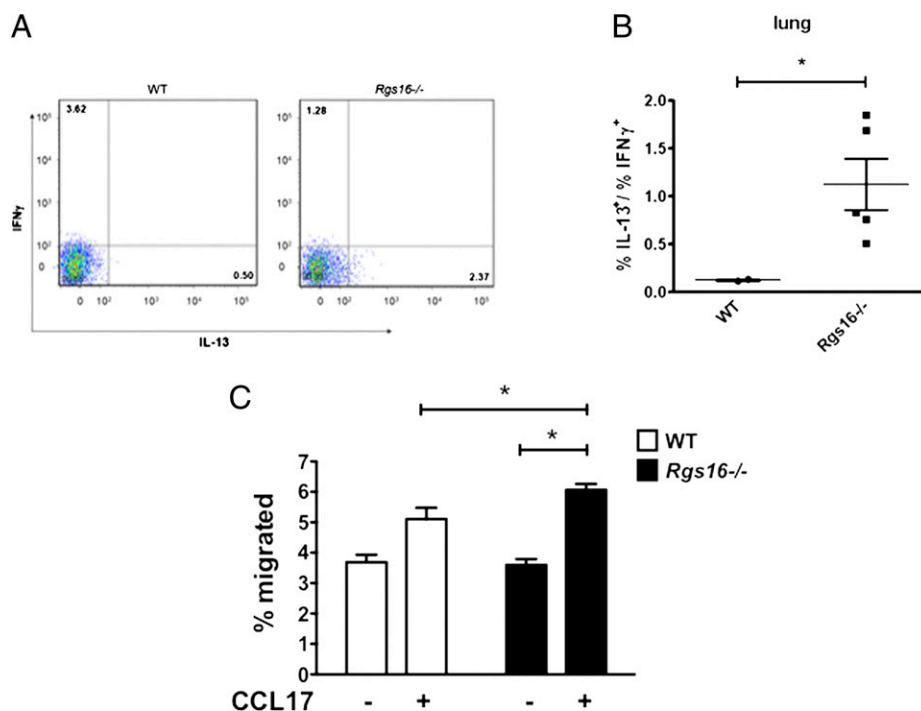


FIGURE 4. Aberrant Th2 migration in *Rgs16*^{-/-} lungs following *S. mansoni* challenge. (**A** and **B**) Frequencies of IL-13⁺ or IFN- γ ⁺ T cells (CD3⁺) (**A**) or ratio IL-13⁺/IFN- γ ⁺ T cell percentages (**B**) in the lungs 4 d following *S. mansoni* challenge were quantified by flow cytometry (**p* = 0.04; unpaired *t* test). The numbers in each quadrant represent a percentage of total T cells. (**C**) Chemotaxis of splenic CD4⁺ T cells collected from the spleens of WT or *Rgs16*^{-/-} mice 4 d following helminth challenge (mean $\pm$ SEM of four to five mice per group; **p* = 0.03; unpaired *t* test).



suggest that RGS16 attenuates inflammation in Schistosome egg-challenged lungs by retaining T lymphocytes within granulomas.

RGS16 regulates lymphocyte trafficking in vivo mediated by CCR4 and CCR10

Because Th2 cells express CCR4, and RGS16-deficient T cells migrated excessively toward CCL17, we hypothesized that the CCL17/22–CCR4 pathway mediated uncharacteristic migration of a subset of Th2 cells (CCR4⁺IL-13⁺) in the challenged lungs of *Rgs16*^{-/-} mice. Consistent with this hypothesis, we detected CCR4⁺IL-13⁺ T cells in the lungs of *Rgs16*^{-/-} much earlier than in WT mice (4 d after challenge with *S. mansoni*) (Fig. 6A). To determine whether other chemokine/receptor pairs contributed to T lymphocyte mislocalization in the lungs of helminth-challenged *Rgs16*^{-/-} mice, we analyzed differential gene expression by qPCR array (full gene list in Supplemental Table I). Notably, we found selectively increased expression of *Il17b* and *Ccr10* mRNA in the lungs of *Rgs16*^{-/-} mice compared with WT. CCR10 is expressed by effector/memory T cells, Langerhans cells, and plasma cells, among others (24–26), and its ligands CCL27/28 are produced by epidermal keratinocytes (27) and displayed on the surface of dermal endothelial cells (28). CCR10 expression on skin-homing Th2 cells (29) has been implicated in the pathogenesis of T cell-mediated inflammatory skin diseases including atopic dermatitis and contact hypersensitivity (26, 27). We detected CCR10 in the lungs of challenged mice by immunohistochemistry, and its staining pattern mirrored T cell localization in WT and *Rgs16*^{-/-} mice (predominantly in granulomas or peribronchial/perivascular areas, respectively) (Fig. 6B). This result suggests a role for CCR10 in *S. mansoni*-evoked inflammation and in the aberrant trafficking of cytokine-producing T cells we observed in *Rgs16*^{-/-} mice.

Although we found decreased or comparable expression of *Ccl17* and CCR10 ligands *Ccl27* and *Ccl28* in the lungs of WT and *Rgs16*^{-/-} mice (in the presence or absence of *S. mansoni* challenge) (Fig. 6C, Supplemental Table I; data not shown), the naive lungs of knockout mice had significantly increased *Ccr10* expression (Fig. 6D). We detected CCR10⁺ T lymphocytes in the naive lungs of *Rgs16*^{-/-} mice but not in WT mice (Fig. 6E),

suggesting that anomalous migration of RGS16-deficient CCR10⁺ T cells to the lungs accounted for the increased *Ccr10* gene expression. Moreover, lungs from *Rgs16*^{-/-} mice, but not from WT

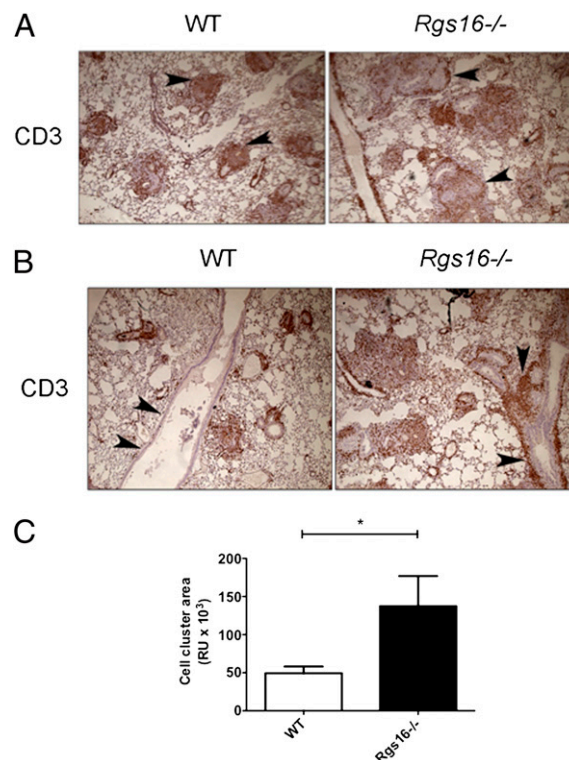


FIGURE 5. Anomalous lymphocyte localization in helminth-challenged *Rgs16*^{-/-} lungs. (**A** and **B**) T cell localization in helminth-challenged lungs evaluated by immunohistochemistry with a CD3 Ab. Images in (**A**) show parenchymal T cell accumulation in granulomas, whereas those in (**B**) demonstrate perivascular/peribronchial cell aggregates (arrowheads). Original magnification $\times 5$. Total area containing cellular aggregates around airways and vessels was quantified using Image Pro Plus software (**p* = 0.04; unpaired *t* test) as indicated in (**C**). Images represent eight mice per group evaluated in two independent experiments.

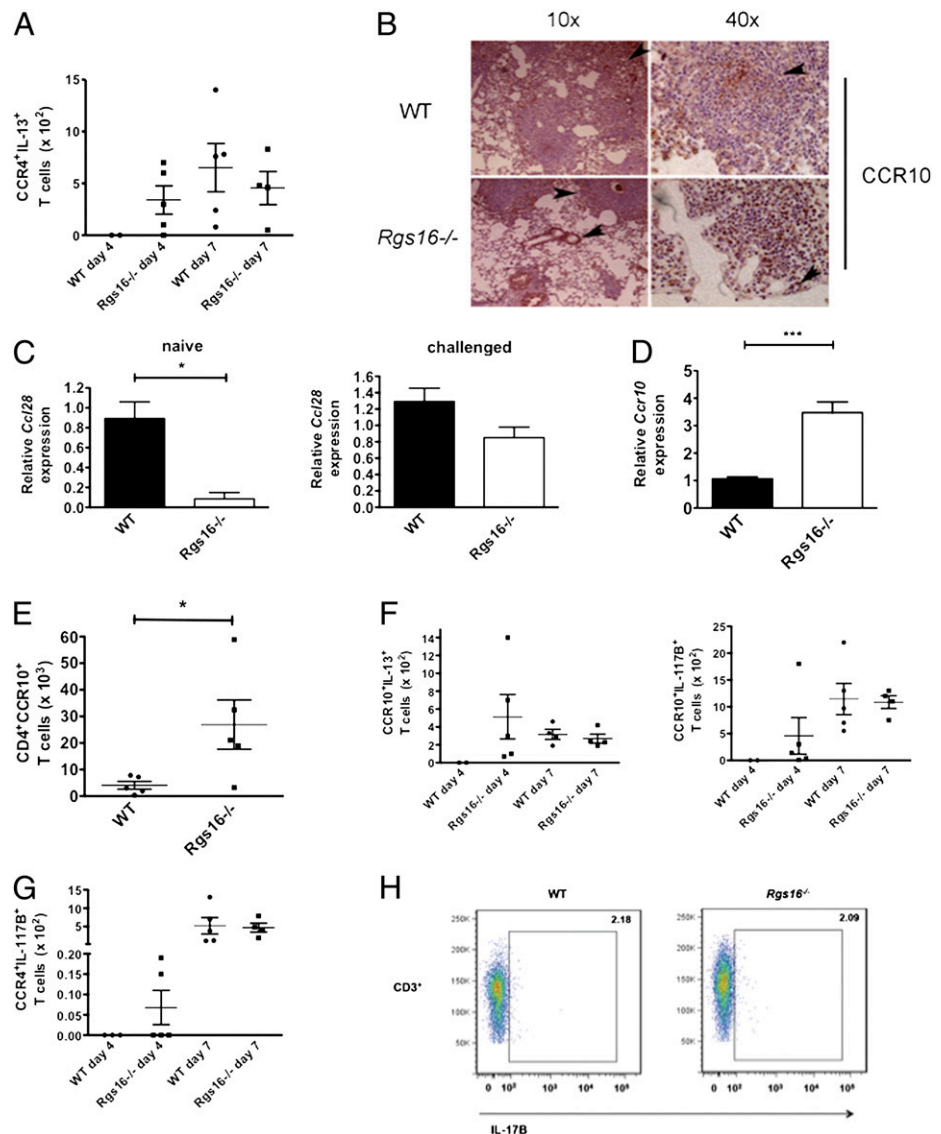


FIGURE 6. Lung lymphocyte populations in *Rgs16*^{-/-} mice following helminth challenge. **(A)** CCR4⁺ Th2 cells (IL-13⁺) in the lungs of challenged mice were enumerated by flow cytometry. **(B)** CCR10 expression in helminth-challenged lungs was evaluated by immunohistochemical staining with a CCR10 Ab. **(C)** and **(D)** *Ccl28* (C) or *Ccr10* (D) expression in naive or *S. mansoni*-challenged lungs evaluated by real-time PCR (**p* = 0.04; unpaired *t* test). **(E–G)** CCR10⁺ T cells in naive lungs (E) or cytokine⁺ CCR10⁺ (F) or CCR4⁺ (G) T cells in helminth-challenged lungs were quantified by means of flow cytometry. **(H)** Frequencies of IL-17B⁺ T cells (CD3⁺) in lungs 7 d following *S. mansoni* challenge were quantified by means of flow cytometry. Numbers represent percentage of total T cells. All data were generated using three naive mice per group, three to five challenged WT mice, or four to five challenged *Rgs16*^{-/-} mice. **p* < 0.05, ****p* < 0.0005.

mice, contained cytokine-producing CCR4⁺ or CCR10⁺ T cells (IL-13 or IL-17B) 4–7 d following *S. mansoni* challenge (Fig. 6F–H). Collectively, these results indicate that ectopic trafficking of CCR4⁺ and CCR10⁺ T effector cells to the lungs of *Rgs16*^{-/-} mice may exacerbate fibrogenesis in response to pulmonary helminth challenge through enhanced cytokine production.

Cytokine abnormalities in *S. mansoni*-challenged *Rgs16*^{-/-} mice

In addition to increased *Ccr10* expression, array analysis also revealed upregulation of cytokines in helminth-challenged *Rgs16*^{-/-} lungs compared with WT—specifically, *Il13* and *Il17b*. We confirmed these results by real-time PCR (Fig. 7A, 7B) and investigated the source(s) of cytokines by restimulating MLN cells from WT or *Rgs16*^{-/-} mice with SEA. LN supernatants from *Rgs16*^{-/-} mice had significantly more IL-5, IL-10, and IL-13 than WT (Fig. 7C). Because effector T cells are generally considered to be the most common sources of these cytokines, we analyzed quantities of LN cytokine-producing CD4 T cells in WT and knockout mice. In contrast to the increased numbers of cytokine⁺ T cells present in the lungs of *S. mansoni*-challenged *Rgs16*^{-/-} mice relative to WT at the earlier time point (4 d postchallenge), frequencies of IL-13⁺ or IL-17B⁺ T cells and the intensity of IL-13 staining were similar in WT and *Rgs16*^{-/-} MLNs at the 7-d time

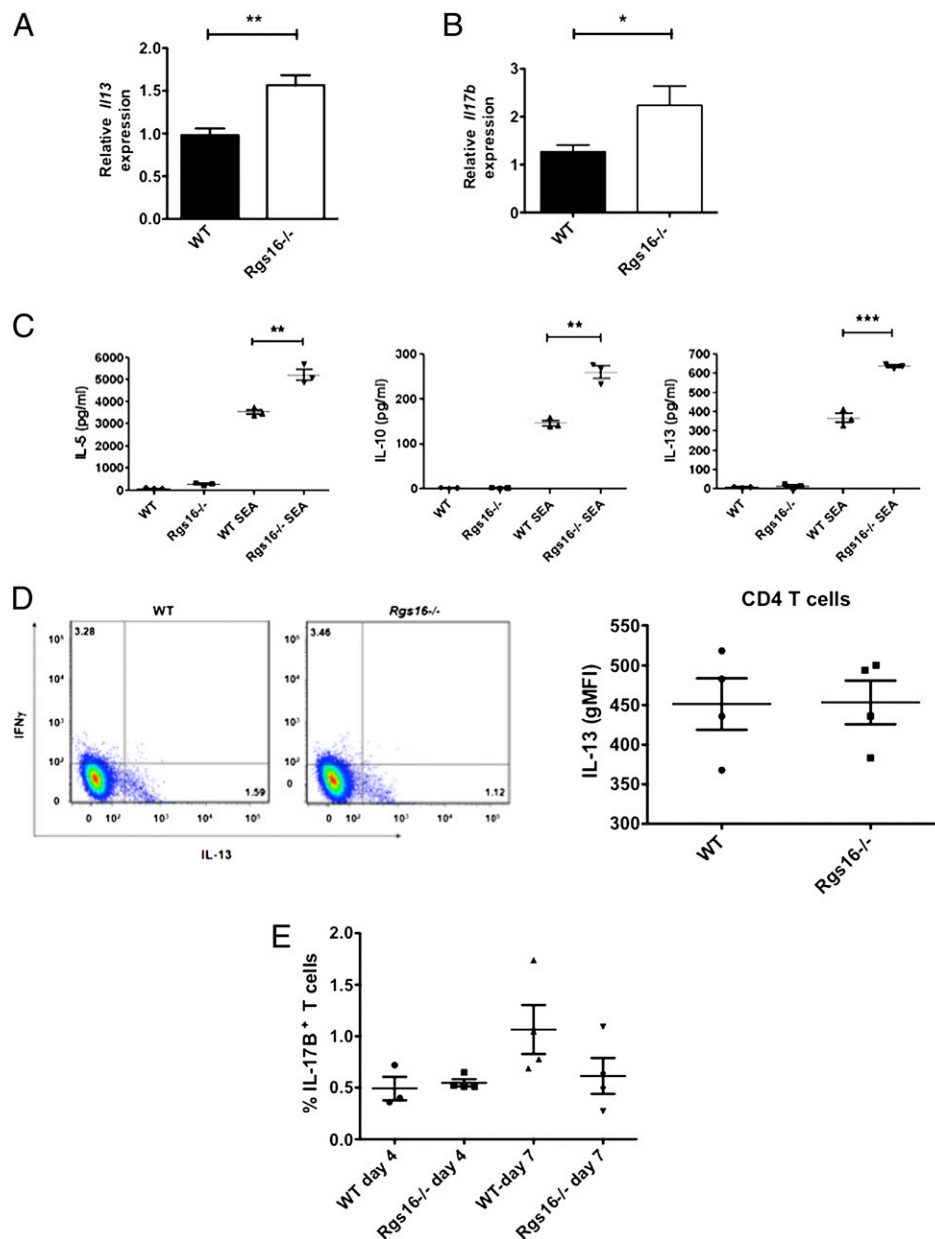
point (Fig. 7D, 7E). These results suggested that further characterization of these cell populations is required to determine the source of increased cytokines in MLN supernatants of *Rgs16*^{-/-} mice relative to WT following re-exposure to *S. mansoni*.

Discussion

We have elucidated a function for a modifier of GPCR signaling, RGS16, in a Th2-mediated murine pulmonary inflammatory response to helminth challenge—specifically, through regulation of lung T cell trafficking and cytokine production. The loss of RGS16 in mice triggered an enhanced granulomatous reaction in the lung following challenge with *S. mansoni* eggs, resulting in more fibrosis and eosinophil influx, anomalous localization of T cells, and increased cytokine production. This study also highlights an unanticipated function of the CCR10–CCL27/8 chemokine axis and IL-17B in the pathogenesis of *S. mansoni*-associated inflammation.

Several lines of evidence suggest that RGS16 directly controls differentiated Th/effector T cell migration patterns but does not regulate trafficking of quiescent, naive T lymphocytes. RGS16 expression is highly upregulated in differentiated mouse and human Th1, Th2, and Th17 cells compared with naive CD4 T cells (Fig. 2A) (13). Chemokines involved prominently in the maintenance of lymphoid compartments through induction of lymphocyte ex-

FIGURE 7. Cytokine abnormalities in *Rgs16*^{-/-} mice inoculated with *S. mansoni*. **(A and B)** Expression of *Il13* (A) or *Il17b* (B) was evaluated in naive or helminth-challenged lungs by real-time PCR (**p* = 0.03, ***p* = 0.005; unpaired *t* test). **(C)** Cells were harvested from lung-draining MLNs 7 d following inoculation with *S. mansoni*. Cells were left untreated or restimulated with SEA for 3 d, followed by measurement of the indicated cytokine levels in supernatants (**p* = 0.005, ****p* = 0.0001; unpaired *t* test). **(D)** Frequencies of IL-13⁺ or IFN- γ ⁺ T cells (CD3⁺) in LNs 7 d following *S. mansoni* challenge were quantified by means of flow cytometry. Numbers in each quadrant represent percentage of total T cells. The graph on the right shows intensity of IL-13 staining (geometric mean fluorescence intensity, MFI) in the CD4 T cell population. **(E)** Frequencies of IL-17B⁺ T cells (CD3⁺) in LNs 4 or 7 d following *S. mansoni* challenge were quantified by means of flow cytometry. All data were generated using three naive mice per group, three to five challenged WT mice, or four to five challenged *Rgs16*^{-/-} mice. For (C), data are representative of seven to eight mice per group evaluated in two independent experiments.



travasation through high endothelial venules of the spleen and LNs (CCL21 and CXCL12) induced comparable chemotaxis of naive WT and RGS16-deficient T cells. In contrast, *Rgs16*^{-/-} effector Th2 lymphocytes differentiated in vitro or cells extracted from *S. mansoni*-challenged lungs had exaggerated chemotaxis toward a Th2-associated chemokine (CCL17). The degree of RGS16 expression correlated inversely with the extent of migration, and chemotaxis of *Rgs16*^{-/-} T cells was reduced by reconstitution with RGS16. Spleen and LN lymphocyte populations and organ architecture in the absence of immune challenge were unchanged in either RGS16-Tg or *Rgs16*^{-/-} mice (Ref. 13 and this study), whereas acute inoculation with *S. mansoni* accentuated trafficking of differentiated Th2 cells to these sites.

A surprising finding of this study is the anomalous collection of CCR10⁺ Th2 cells in the lungs of *Rgs16*^{-/-} mice. Although the CCR10–CCL27 axis has been previously associated with Th2-mediated inflammation in the skin, its role in pulmonary pathology induced by *S. mansoni* challenge was unknown (20). CCL27 is produced in skin epidermal keratinocytes (27) and presented by dermal endothelial cells (28). CCL17 also promotes CCL27 in-

duction by keratinocytes in the presence of TNF- α (30). CCL28, another CCR10 ligand, has been implicated in CCR10-mediated leukocyte homing to the respiratory tract in a murine asthma model (31). The presence of similar or reduced amounts of CCL27/28 ligands in the lungs of *Rgs16*^{-/-} mice compared with WT suggests that aberrant chemotactic responses of RGS16-deficient T cells to these ligands underlies their accumulation in lungs in the absence of immune challenge. However, for unknown reasons, we did not observe chemotaxis of Th2 cells (WT or knockout) toward these chemokines in Transwell assays in vitro despite expression of CCR10.

We also found increased *Il13* expression in the challenged lungs of *Rgs16*^{-/-} mice relative to WT, consistent with its central contribution to fibrosis induced by helminth infection (32–33). Although previous work has also defined a role for IL-17A in murine lung inflammation (34), we observed increased *Il17b* expression in the lungs of *S. mansoni*-challenged *Rgs16*^{-/-} mice relative to WT but did not detect *Il17a*. These results suggest an unanticipated function of IL-17B in helminth immunity. Among other IL-17 family members, neutralization of IL-17B in a collagen-induced

model of murine arthritis suppressed disease progression by reducing cell infiltration and production of proinflammatory cytokine such as IL-1 β and TNF- α (35), factors also known to induce CCL28 in airway epithelial cells (36, 37). Although we detected IL-17B⁺ T cells in the challenged lungs, it is unclear whether these cells are conventional Th17 cells. Indeed, in vitro-differentiated Th17 cells produced IL-17A but not IL-17B (Supplemental Fig. 3). Taken together, these findings suggest the presence of a unique proinflammatory environment downstream of IL-17B in the lungs of *Rgs16*^{-/-} mice challenged with *S. mansoni*. The absence of RGS16 may promote T cell chemotaxis to CCL27/28 displayed on the surface of endothelial cells, which could account for the peribronchial/perivascular accumulation of T cells we observed in the challenged lungs of *Rgs16*^{-/-} mice.

How RGS16 regulates Ag-induced cytokine production requires further study. We detected unique populations of IL-13 or IL-17B cytokine-producing cells expressing CCR10 or CCR4 much earlier in the lungs of *Rgs16*^{-/-} mice following helminth challenge. Although quantities of cytokine⁺ T cells in the lungs were equivalent in the two strains by day 7, overall lung cytokine expression was increased in the lungs of knockout mice at this time point relative to WT. Additional gene array analysis revealed reduced expression of *Socs2* in the lungs of naive *Rgs16*^{-/-} mice compared with WT counterparts but not in RGS16-deficient Th2 cells differentiated in vitro (Supplemental Fig. 3; data not shown). These data suggest that lung T cells in *Rgs16*^{-/-} mice are primed for increased Th2 cytokine production as a result of reduced *Socs2* expression (38).

In contrast, although levels of immunoreactive cytokines in the supernatants of Ag-restimulated MLN cells extracted from *Rgs16*^{-/-} mice 7 d post-*S. mansoni* challenge were significantly higher than those from WT mice, flow cytometric analysis demonstrated equivalent cytokine⁺ T cell frequencies and intensity of cytokine staining in these same LN T cell populations. Polarized Th cells from WT and *Rgs16*^{-/-} mice secreted roughly equivalent cytokine amounts (IFN- γ for Th1, IL-4 or IL-13 for Th2, and IL-17A for Th17) in response to TCR stimulation with anti-CD3 and anti-CD28 in vitro (Supplemental Fig. 3; data not shown). Increased accumulation of specific populations of cytokine-producing Th cells in MLNs of *Rgs16*^{-/-} mice compared with WT (presumably because of altered trafficking patterns) could also account for the overall increases in secreted cytokines observed. Thus, on the basis of these data alone, we cannot yet determine whether Ag-stimulated, RGS16-deficient T lymphocytes generate more cytokine than WT cells on a per cell basis.

Although our work and that of others have shown that RGS proteins inhibit chemokine-mediated lymphocyte chemotaxis and adhesive responses in vitro (39, 40), function(s) of RGS proteins in T cell-mediated immunity have not been explored in detail. Surprisingly, *Rgs2*^{-/-} mice had reduced footpad swelling following local inoculation with lymphocytic choriomeningitis virus compared with WT, which correlated with impaired proliferation of, and IL-2 production by, RGS2-deficient T cells. Taken together, these results and our studies suggest unique, context-dependent functions of individual RGS proteins in immune cells that may or may not be predictable, based on their shared biochemical (GAP) activity. Further exploration of RGS16 in specific T cell populations and in the setting of immune challenges will be needed to fully clarify its function(s) in adaptive immunity. Although we present abundant evidence that anomalous Th2 trafficking and increased T cell-derived cytokines contribute to the enhanced pulmonary inflammation in *S. mansoni*-challenged, RGS16-deficient mice relative to WT (Figs. 4–7), non-Th-dependent factors may also mediate fibrosis in this setting. Ad-

ditional models of inflammation and approaches such as adoptive T cell transfer and/or generation of bone marrow chimeras will be needed to determine the relative importance of T cell-intrinsic and T cell-independent factors to the immune responses of mice lacking RGS16.

Disclosures

The authors have no financial conflicts of interest.

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Supplemental Figures

Fig. S1

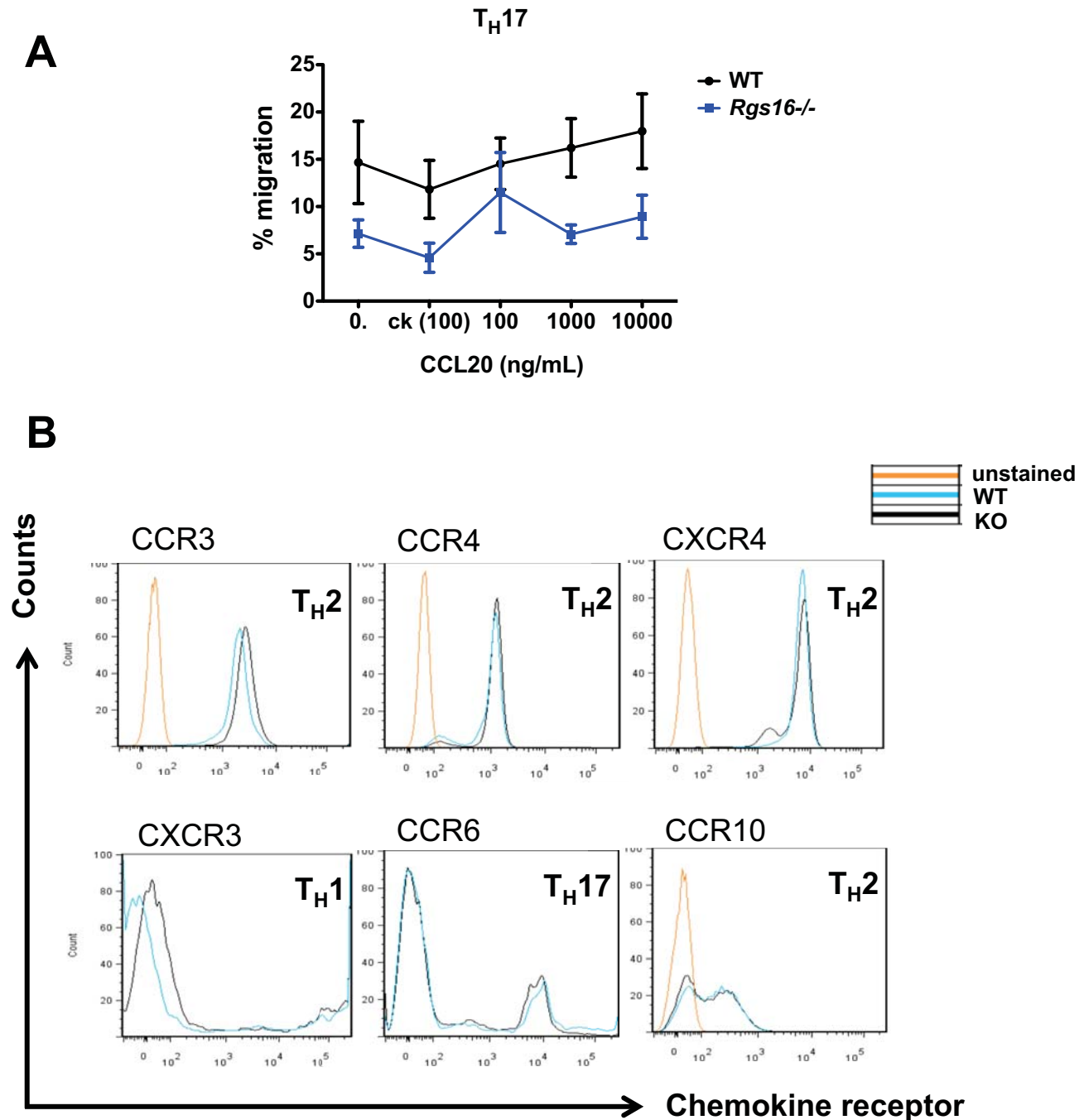
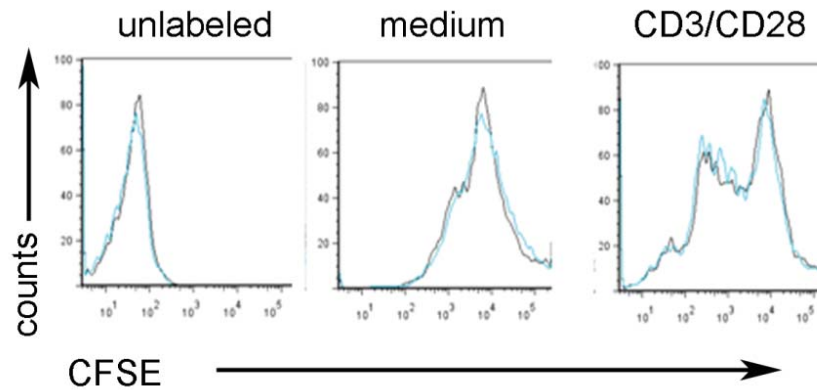


Fig. S1. Chemotaxis of *in vitro*-differentiated T_H17 cells and chemokine receptor expression in T_H cells. (A) T_H17 cells were differentiated from naïve LN cells of WT or *Rgs16*^{-/-} mice as per the Methods. Chemotaxis of T_H17 cells was determined in transwell assays as in Fig. 2. Graph shows mean ± S.E.M. of 2 experiments using cells pooled from LNs of 2 mice of each genotype. (B) T_H1, T_H2, or T_H17 cells were differentiated from naïve LN cells of WT or *Rgs16*^{-/-} mice as per the Methods. Chemokine receptor expression was determined by means of flow cytometry. Graph shows results of a single experiment representative of 2-3 experiments using cells pooled from LNs of 2-3 mice of each genotype.

Fig. S2

A



B

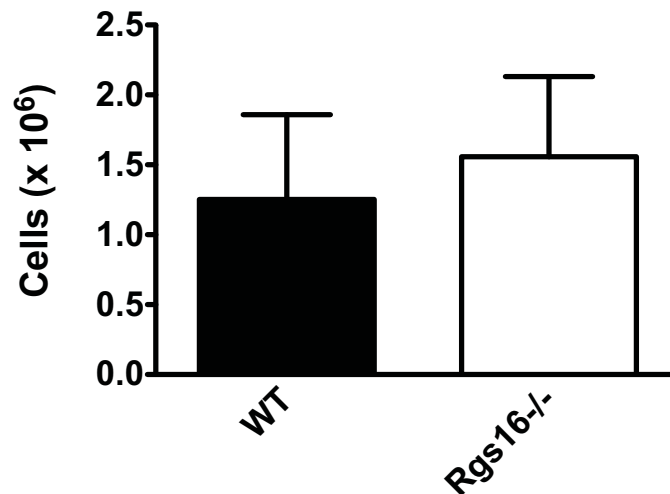


Fig. S2. Intact proliferation and development RGS16-deficient T_H cells. (A) T_H2 cells were differentiated from naïve LN cells of WT or *Rgs16*^{-/-} mice as per the Methods. Proliferation of untreated (middle) or anti-CD3/anti-CD28-treated (right) cells was determined by means of CFSE labeling and flow cytometry. (B) Differentiated T_H2 cells WT or *Rgs16*^{-/-} mice were quantified after a 3 day incubation in basal medium. Graphs shows results of a single experiment representative of 3 experiments using cells pooled from LNs of 1 mouse of each genotype.

Fig. S3

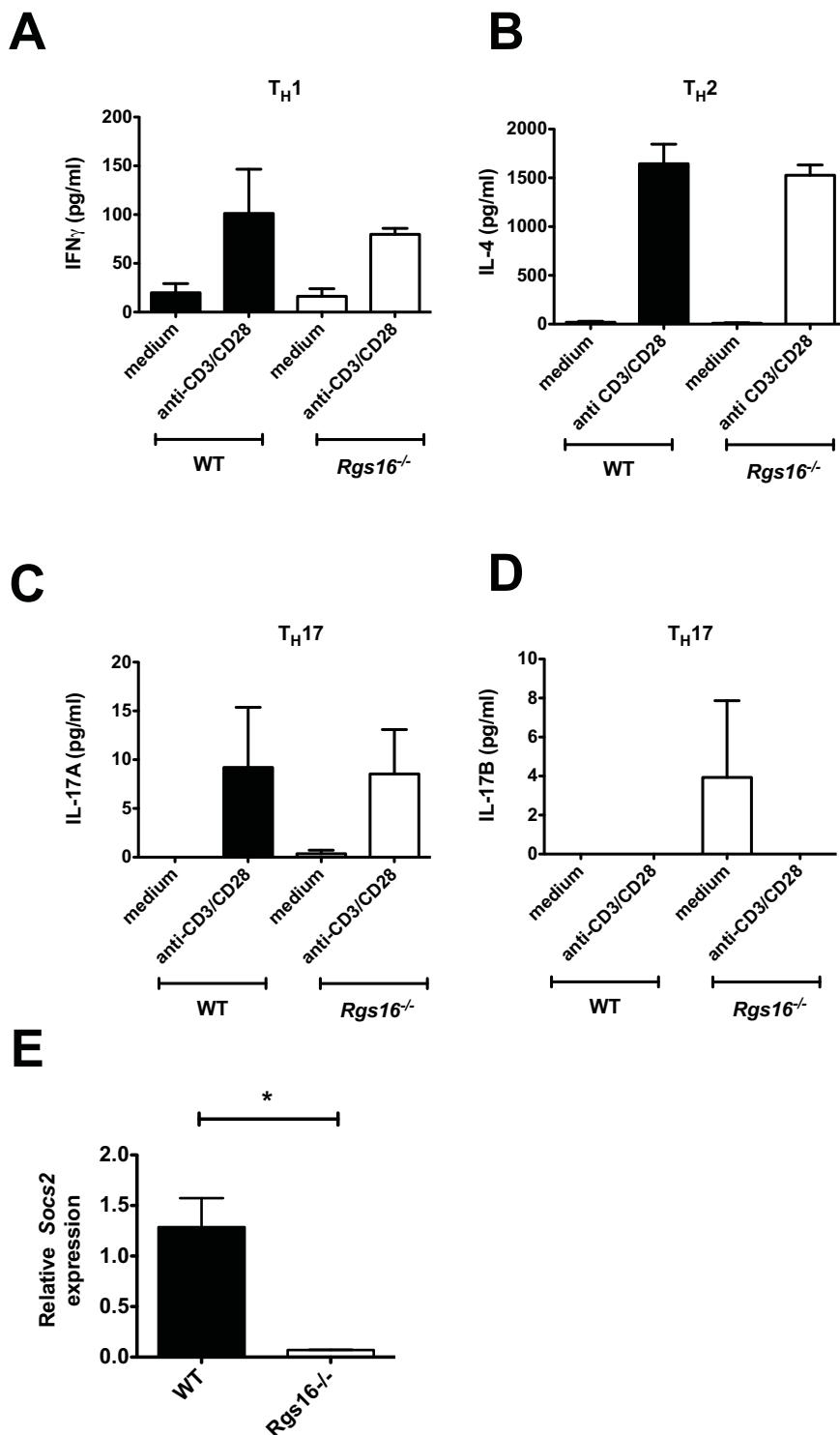


Fig. S3. RGS16 deficiency does not impair cytokine production by T_H cells. (A-D) Naïve T cells from LNs of WT or *Rgs16*^{-/-} mice were differentiated *in vitro* as outlined in the Methods. Polarized T_H cells were left untreated or treated with anti-CD3 and anti-CD28 antibodies for 3 days. Cytokine levels in supernatants were quantified by means of ELISA. (E) *Socs2* gene expression was quantified in lungs of naïve WT or *Rgs16*^{-/-} mice by means of qPCR. Graphs portrays results (mean $\pm$ S.E.M) obtained from 2 mice of each genotype (* $P \leq 0.05$, *t* test).

Table S1

Description	Symbol	fold change <i>Rgs16</i>^{-/-} v. WT
ATP-binding cassette, sub-family F (GCN20), member 1	Abcf1	1.1694
B-cell leukemia/lymphoma 6	Bcl6	1.0972
Chemokine (C-X-C motif) receptor 5	Cxcr5	-1.1213
Complement component 3	C3	1.0145
Caspase 1	Casp1	1.0672
Chemokine (C-C motif) ligand 1	Ccl1	-1.3372
Chemokine (C-C motif) ligand 11	Ccl11	1.0265
Chemokine (C-C motif) ligand 12	Ccl12	-1.2271
Chemokine (C-C motif) ligand 17	Ccl17	1.4497
Chemokine (C-C motif) ligand 19	Ccl19	1.6734
Chemokine (C-C motif) ligand 2	Ccl2	-1.2186
Chemokine (C-C motif) ligand 20	Ccl20	-1.005
Chemokine (C-C motif) ligand 22	Ccl22	-1.2546
Chemokine (C-C motif) ligand 24	Ccl24	-1.173
Chemokine (C-C motif) ligand 25	Ccl25	1.1002
Chemokine (C-C motif) ligand 3	Ccl3	1.1071
Chemokine (C-C motif) ligand 4	Ccl4	-1.3097
Chemokine (C-C motif) ligand 5	Ccl5	-1.2169
Chemokine (C-C motif) ligand 6	Ccl6	1.2404
Chemokine (C-C motif) ligand 7	Ccl7	-1.0944
Chemokine (C-C motif) ligand 8	Ccl8	1.1272
Chemokine (C-C motif) ligand 9	Ccl9	1.3166
Chemokine (C-C motif) receptor 1	Ccr1	-1.1844
Chemokine (C-C motif) receptor 2	Ccr2	-1.1844
Chemokine (C-C motif) receptor 3	Ccr3	-1.1291
Chemokine (C-C motif) receptor 4	Ccr4	-1.2677
Chemokine (C-C motif) receptor 5	Ccr5	-1.3316
Chemokine (C-C motif) receptor 6	Ccr6	1.4052
Chemokine (C-C motif) receptor 7	Ccr7	1.1932
Chemokine (C-C motif) receptor 8	Ccr8	-1.1577
Chemokine (C-C motif) receptor 9	Ccr9	1.7481
C-reactive protein, pentraxin-related	Crp	1.1469
Chemokine (C-X3-C motif) ligand 1	Cx3cl1	1.3093
Chemokine (C-X-C motif) ligand 1	Cxcl1	-1.0922
Chemokine (C-X-C motif) ligand 10	Cxcl10	-1.4694
Chemokine (C-X-C motif) ligand 11	Cxcl11	-1.0794
Chemokine (C-X-C motif) ligand 12	Cxcl12	1.1265
Chemokine (C-X-C motif) ligand 13	Cxcl13	1.0724
Chemokine (C-X-C motif) ligand 15	Cxcl15	1.0173
Platelet factor 4	Pf4	1.0366
Chemokine (C-X-C motif) ligand 5	Cxcl5	1.2877
Chemokine (C-X-C motif) ligand 9	Cxcl9	-1.7928
Chemokine (C-X-C motif) receptor 3	Cxcr3	1.417
Chemokine (C-C motif) receptor 10	Ccr10	3.215
Interferon gamma	Ifng	-1.2898
Interleukin 10	Il10	1.0591
Interleukin 10 receptor, alpha	Il10ra	1.1079
Interleukin 10 receptor, beta	Il10rb	1.1808
Interleukin 13	Il13	-1.1738

Interleukin 13 receptor, alpha 1	Il13ra1	1.0047
Interleukin 15	Il15	1.5635
Interleukin 16	Il16	1.1686
Interleukin 17B	Il17b	4.2216
Interleukin 18	Il18	1.0664
Interleukin 1 alpha	Il1a	1.0746
Interleukin 1 beta	Il1b	-1.0226
Interleukin 1 family, member 6	Il1f6	-1.063
Interleukin 1 family, member 8	Il1f8	-1.4858
Interleukin 1 receptor, type I	Il1r1	1.0746
Interleukin 1 receptor, type II	Il1r2	1.3792
Interleukin 2 receptor, beta chain	Il2rb	-1.2511
Interleukin 2 receptor, gamma chain	Il2rg	-1.0376
Interleukin 3	Il3	1.5744
Interleukin 4	Il4	-1.0268
Interleukin 5 receptor, alpha	Il5ra	-1.1918
Interleukin 6 receptor, alpha	Il6ra	-1.0134
Interleukin 6 signal transducer	Il6st	1.018
Interleukin 8 receptor, beta	Il8rb	-1.4066
Integrin alpha M	Itgam	1.1343
Integrin beta 2	Itgb2	1.1002
Lymphotoxin A	Lta	-2.2724
Lymphotoxin B	Ltb	-1.052
Macrophage migration inhibitory factor	Mif	-1.0311
Small inducible cytokine subfamily E, member 1	Scye1	1.3138
Secreted phosphoprotein 1	Spp1	1.2976
Transforming growth factor, beta 1	Tgfb1	-1.0922
Tumor necrosis factor	Tnf	1.1581
Tumor necrosis factor receptor superfamily, member 1a	Tnfrsf1a	1.1565
Tumor necrosis factor receptor superfamily, member 1b	Tnfrsf1b	1.5766
CD40 ligand	Cd40lg	1.2612
Toll interacting protein	Tollip	1.1079
Chemokine (C motif) receptor 1	Xcr1	-1.0645

Supplemental Table 1. Differential gene expression was analyzed in total RNA from challenged lungs of *Rgs16*^{-/-} or WT C57/Bl5 mice by qPCR array. Data represent pooled RNA from 5 WT or 4 knockout mice; fold changes greater than 2 were considered significant.