Follicular Dendritic Cell Regulation of CXCR4-Mediated Germinal Center CD4 T Cell Migration

Jacob D. Estes, Tyler C. Thacker, Denise L. Hampton, Sariah A. Kell, Brandon F. Keele, Emily A. Palenske, Kirk M. Druey and Gregory F. Burton

*J Immunol* 2004; 173:6169-6178; doi: 10.4049/jimmunol.173.10.6169
http://www.jimmunol.org/content/173/10/6169

**References**

This article cites 65 articles, 32 of which you can access for free at: http://www.jimmunol.org/content/173/10/6169.full#ref-list-1

**Subscription**

Information about subscribing to *The Journal of Immunology* is online at: http://jimmunol.org/subscription

**Permissions**

Submit copyright permission requests at: http://www.aai.org/About/Publications/JI/copyright.html

**Email Alerts**

Receive free email-alerts when new articles cite this article. Sign up at: http://jimmunol.org/alerts

*The Journal of Immunology* is published twice each month by The American Association of Immunologists, Inc., 1451 Rockville Pike, Suite 650, Rockville, MD 20852. Copyright © 2004 by The American Association of Immunologists All rights reserved. Print ISSN: 0022-1767 Online ISSN: 1550-6606.
Follicular Dendritic Cell Regulation of CXCR4-Mediated Germinal Center CD4 T Cell Migration

Jacob D. Estes,* Tyler C. Thacker,* Denise L. Hampton,‡ Sariah A. Kell,§ Brandon F. Keele,* Emily A. Palenske,* Kirk M. Druey,‡ and Gregory F. Burton2†

Follicular dendritic cells (FDCs) up-regulate the chemokine receptor CXCR4 on CD4 T cells, and a major subpopulation of germinal center (GC) T cells (CD4+CD57+), which are adjacent to FDCs in vivo, expresses high levels of CXCR4. We therefore reasoned that GC T cells would actively migrate to stromal cell-derived factor-1 (CXCL12), the CXCR4 ligand, and tested this using Transwell migration assays with GC T cells and other CD4 T cells (CD57−) that expressed much lower levels of CXCR4. Unexpectedly, GC T cells were virtually nonresponsive to CXCL12, whereas CD57−CD4 T cells migrated efficiently despite reduced CXCR4 expression. In contrast, GC T cells efficiently migrated to B cell chemotactrant-1/CXCL13 and FDC supernatant, which contained CXCL13 produced by FDCs. Importantly, GC T cell nonresponsiveness to CXCL12 correlated with high ex vivo expression of regulator of G protein signaling (RGS), RGS13 and RGS16, mRNA and expression of protein in vivo. Furthermore, FDCs up-regulated both RGS13 and RGS16 mRNA expression in non-GC T cells, resulting in their impaired migration to CXCL12. Finally, GC T cells down-regulated RGS13 and RGS16 expression in the absence of FDCs and regained migratory competence to CXCL12. Although GC T cells express high levels of CXCR4, signaling through this receptor appears to be specifically inhibited by FDC-mediated expression of RGS13 and RGS16. Thus, FDCs appear to directly affect GC T cell migration within lymphoid follicles. The Journal of Immunology, 2004, 173: 6169–6178.

Combating invading pathogens efficiently requires a series of orchestrated movements of T and B lymphocytes as well as APCs to and within the secondary lymphoid tissues (1). Chemokines are important proteins responsible for guiding lymphocytes into lymphoid organs and compartmentalization within these sites (1, 2). Naïve B and T cells enter the lymph node through high endothelial venules (3–5), where the secondary lymphoid tissue chemokine (CCL21) and the stromal cell-derived factor-1 (CXCL12) chemokine are responsible for the induction of firm arrest of rolling T and B lymphocytes, respectively (6–9). After extravasation, naive lymphocytes are attracted to the para-cortical region of the parenchyma via CCL21 and EBI 1-ligand chemokine (CCL19) and CXCL12 (10).

Chemokines bind to receptors that belong to a large family of seven-transmembrane, G protein-coupled receptors on the surface of leukocytes (11). Chemokine receptors are coupled to heterotrimeric Gαβγ proteins. Agonist binding to the receptor catalyzes the exchange of GTP for GDP on the Gα subunit and induces dissociation of the Gα and Gβγ subunits (reviewed in Ref. 11). The GTP-bound Gα subunit and the Gβγ subunits independently activate multiple downstream effectors, one result of which is cell migration (reviewed in Ref. 12). However, chemokine binding to its cognate receptor(s) does not always result in cell migration. Bleul et al. (13) showed that germinal center (GC)3 B cells, which express high levels of surface CXCR4, failed to migrate in response to CXCL12 and that receptor desensitization may serve as a control mechanism of cell migration. Recently, the regulator of G protein signaling (RGS) family of proteins was discovered and found to associate with specific Gα subunits, markedly stimulating (100- to 1000-fold) their native GTPase activity. As a result, the signaling potential of the G protein-coupled receptor is inhibited (reviewed in Ref. 14). Because chemokine receptor signaling is critical in directing cells to and within tissue compartments, localized control of RGS expression could contribute to receptor desensitization and prevent cells from migrating despite continued expression of receptors and exposure to chemokines (15).

A large body of work on the generation of thymus-dependent (TD) Ab responses suggests that Ag-signaled B cells initially undergo cognate interactions with T lymphocytes at the edge of the lymphoid follicles in secondary lymphoid tissue (16–20). Some B cells differentiate at this stage into short-lived, Ab-forming cells, but other activated Ag-specific B and T cells migrate into the follicles where they form GCs surrounding follicular dendritic cells (FDCs) (16–18).

FDCs are restricted to the follicles of secondary lymphoid tissue (e.g., lymph nodes, spleen, and tonsils) and are thought to play a key role in the formation of GCs as well as in the selection, differentiation, and maintenance of memory B cells (21–23). Their long cytoplasmic extensions form a reticular network throughout lymphoid follicles that traps and retains Ags in the form of immune complexes (24–27). FDC-trapped Ags persist for many months in an unprocessed form and serve to maintain long-term, memory IgG and IgE responses to soluble protein Ags (21, 24, 25).

Copyright © 2004 by The American Association of Immunologists, Inc.

1 Abbreviations used in this paper: GC, germinal center; ABS, Ab binding site; FDC, follicular dendritic cell; RGS, regulator of G protein signaling; TD, thymus dependent.
During humoral immune responses, FDCs play a critical role as accessory cells, presenting Ag-Ab complexes to B cells (27, 30). FDCs also provide signals to T and B lymphocytes that alter their state of activation/proliferation (31, 32) and render B cells responsive to chemotacticants (33). In addition, we have recently shown that FDCs up-regulate the chemokine receptor, CXCR4, on CD4 T cells in vitro and that CD4^+CD57^- GC T cells, a major population of GC T cells, which interact with FDCs in vivo (34), express high levels of CXCR4 compared with other CD4 T cells (CD57^-) (35). Thus, in normal physiology, FDCs play an important role in the GC reaction that generates and maintains TD humoral immune responses.

Naive and resting B cells express the chemokine receptor CXCR5, which is required for the development of follicles in some secondary lymphoid tissues as well as for B cell localization in the follicles of spleen and Peyer’s patches (36). CXCR5 is also expressed on a subset of circulating human memory CD4 T cells (37) and is up-regulated on some mouse CD4 T cells after immunization (38). On murine T cells, CXCR5 regulation appears to require CD28-mediated, OX40 signaling (39). Recently, a novel subpopulation of B helper-T cells that are localized within lymphoid follicles has been defined and termed follicular B helper T cells or germinal center Th cells (GC-Th) (40, 41). These CD4 T cells express the CXCR5 chemokine receptor, are CD57^+, and reside in the GCs of secondary lymphoid tissues, where they produce elevated levels of IL-10 on stimulation and support the efficient production of IgG and IgA. Importantly, these GC T cells were found to interact not only with GC B cells, but also with FDCs, as demonstrated by their direct contact with FDCs in vivo (34).

FDCs, in addition to their role in the development and maintenance of the GC reaction, may play a key role in recruiting both B and T cells into the lymphoid follicle to initiate the GC reaction. In support of this hypothesis, in situ hybridization analysis indicates that B cell chemotrajectant-1 (CCL13), a CXC chemokine that attracts CXCR5^+ cells, is constitutively expressed by resident stromal cells in the GC (1, 42–44). These latter cells are most likely a subset of FDCs; however, it has not been shown that isolated FDCs produce CCL13. An understanding of chemokines and their interactions with specific receptors on motile B and follicular B helper T cells provides an explanation of how these cells come into contact with stationary FDCs to initiate the GC reaction (42).

In the present study, we examined the contributions of FDCs to GC T cell migration, and found that although GC T cells express high levels of CXCR4, they were specifically nonresponsive to CXCL12-induced migration. Such nonresponsiveness correlated with FDC mediated up-regulation of RGS13 and RGS16 expression in CD4 T cells. Additionally, we show that FDCs express and produce CXCL13, which specifically attracts GC T cells via CXCR5. FDC regulation of GC T cell responsiveness to CXCL12 may play an important role in recruiting and retaining Ag-specific cells within the GC to induce and maintain the GC reaction, which results in the differentiation of Ag-specific B cells into Ab-forming cells and the generation of B memory cells (45).

Materials and Methods

Flow cytometric analysis

Cell surface Ags were detected using the following mAbs: anti-human CD4-PeCy5 (13B8.2), anti-human CD14-PE (RM052), anti-human CD21-FITC (BL13), anti-human CD45RO-PE (UCHL1), anti-human CD57 conjugated to FITC or biotin (NC1), and anti-CD69-PE (TPI, 55.3, Immunotech, Westbrook, ME); anti-human CXCR4-PE (1205; BD Biosciences, San Jose, CA); mouse IgM and anti-human Fc (H2J; gift from Dr. M. Nahm, University of Alabama, Birmingham, AL); mouse IgG1 and anti-human CD21L (7D6; gift from Dr. Y. J. Liu, DNAX, Palo Alto, CA); and donkey, F(ab')2, anti-mouse IgM-FITC (Jackson ImmunoResearch Labo-

FDC isolation

Human FDCs were isolated from tonsillar tissue as previously described (46). FDC-enriched preparations prepared using this procedure were examined by flow cytometry and typically contained 75–90% FDCs with residual cells consisting of T and B lymphocytes. In addition, in some experiments FDCs were FACS-purified using a FACSsoultion VSE equipped with the FACSDiVa option and software (BD Biosciences, San Jose, CA). Briefly, low density tonsillar cells were collected from continuous Percoll gradients after centrifugation, washed, and incubated with heat-aggregated, human ChromePure IgG (Jackson Immunoresearch Laboratories) to block nonspecific FcR binding, labeled with H2J (250 μl of hydridoma supernant) and 7D6 (250 μl of hydridoma supernant) on ice for at least 1 h, followed by washing and addition of goat F(ab')2, anti-mouse IgM-FITC (μ-chain specific; 25 μg) and goat F(ab')2, anti-mouse IgG-PE (γ-chain specific; 25 μg). FDCs were sorted on H2J2^-/7D6^- events (this population typically ranged from 0.5–3% of the total population post-Percoll). In all coculture experiments, an FDC to CD4 T cell ratio of 1:10 was used, because we found this to result in optimal FDC-lymocyte interactions (35). In some experiments FDCs were specifically depleted by collecting the effluent from MACS columns used to positively select FDCs, and these cells were then subjected to an additional round of depletion using H2J and magnetic beads (rat, anti-mouse IgM Dynabeads; Dynal Biotech, Great Neck, NY) at a concentration of 10 beads per target cell. This treatment removed ≥ 90% of the FDCs.

CD4 T cell preparations

CD4^+CD57^- GC T cells were isolated from human tonsillar tissue as previously described (35). Briefly, tonsils were cut into small sections, cells were mechanically separated from tissue by repeated pipetting, and RBCs were removed by incubation for 5 min at room temperature in RBC-lysis buffer (155 mM NaHCO3, 10 mM KHCO3, and 0.1 mM EDTA). GC CD4 T lymphocytes were purified by negative selection using a CD4^+ T cell isolation kit (Miltenyi Biotec, Auburn, CA), followed by positive selection by MACS using anti-CD57-biotin and streptavidin microbeads (Miltenyi Biotec). The resulting CD4^+CD57^- preparations were ≥ 95% pure as assessed by flow cytometry.

Chemotaxis assay

Cell migration was evaluated using a 24-well, 5-μm pore size Transwell system (Costar, Cambridge, MA). Purified cells were washed once in chemotaxis medium (RPMI 1640 containing HEPES buffer (20 mM) and gentamicin (50 μg/ml; Invitrogen Life Technologies, Gaithersburg, MD) and then added to 5 × 10^5 cells/ml of chemotaxis medium. An aliquot (100 μl) of the above cell suspension containing 5 × 10^5 cells was placed on the top of the Transwell. Chemokines, prepared at the indicated concentrations (determined by titration assay) in chemotaxis medium (600–1 μl total volume), were added to the bottom of the Transwell system. After 4- to 5-h incubation at 37°C in a 5% CO2 atmosphere, the inserts were removed, and the number of cells that had migrated into the lower well was analyzed by counting cells for 60 s on an EPICS XL flow cytometer (Beckman Coulter, Fullerton, CA). This treatment removed ≥ 90% of the cells migrating through were labeled with anti-CD57-FITC and counted as described above. To establish the number of cells that migrated nonspecifically, the migration assays were performed in parallel in the absence of chemotacticant. Results are expressed as percent specific migration, which was calculated as follows: (total number of cells migrating in the presence of chemokine minus the total number of cells migrating in the absence of chemokine) + (the total number of input cells) × 100.
RNA isolation and RT-PCR analysis

Immediately upon isolation or in some cases after cell culture, an equal number of CD4 T cells was centrifuged and resuspended in PBS (100-μl total volume), and RNA STAT 60 (Tel-Test, Friendswood, PA) was added at a ratio of 800 μl of RNA-RNASTAT60 per 1.0 × 10^6 cells in sterile, 1.5-ml Eppendorf tubes, which were stored at −80°C until use or at −20°C for 10 days. Each Eppendorf tube was vortexed and then centrifuged at 13,000 × g for 15 min at 4°C. An equal volume of the aqueous phase (400 μl) from each sample was transferred to a fresh tube containing the same volume of isopropanol, and the contents were incubated at −20°C for 30 min, and centrifuged at 13,000 × g for 30 min at 4°C. After discarding the supernatant fluid, the RNA pellet was washed once in 75% ethanol (1.0 ml) and air-dried, and the RNA was resuspended using the GeneAmp RNA PCR kit (Roche Molecular Systems, Branchburg, NJ) according to the manufacturer’s instructions. The resulting cDNA was then PCR-amplified under the following conditions: one cycle at 94°C for 50 s, followed by 30 cycles of 94°C for 30 s, 58°C for 1 min, 72°C for 1 min, and a single 10-min extension cycle at 72°C (47). In some instances (i.e., RGS1, -13, and -16), PCR analysis was performed for 26, 28, and 30 cycles on aliquots of sample cDNA to ensure that analysis occurred during the linear phase of the amplification process so that subtle differences in concentration would be detected. Densitometric analysis of the resulting amplicons was performed using National Institutes of Health ImageJ software (Bethesda, MD). Pretreatment of RNA with DNA-free DNase (Ambion, Austin, TX) was performed according to the manufacturer’s instructions. Briefly, mouse, anti-human CXCL13 mAb (2 μg/ml; NeoMarkers, Fremont, CA) and rabbit, affinity-purified RGS13 (provided by Dr. J. H. Kehrl, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD), rabbit, affinity-purified RGS16-210, or preimmune rabbit IgG polyclonal Abs were used at 1 μg/ml in TNB with 5% normal goat serum. Detection of the rabbit primary Abs (anti-human RGS13 and RGS16, and preimmune rabbit IgG) was performed after incubation with biotinylated goat anti-rabbit IgG Ab (4 μg/ml; Vector Laboratories) for 30 min at room temperature, followed by incubation with alkaline phosphatase-labeled avidin-biotin reagent (ABC Elite kit; Vector Laboratories) for 30 min and room temperature and visualized using Vector Red as the substrate for 30 min in a humid chamber at room temperature in the dark. Detection of the mouse anti-human CD57 primary Ab was performed after incubation with biotinylated goat anti-mouse IgM (μ-chain specific) Ab (1 μg/ml; Vector Laboratories) for 30 min at room temperature, followed by incubation with HRP-labeled avidin-biotin reagent (ABC Elite kit) for 30 min at room temperature and visualized with Vector SG (bluish-gray color; Vector Laboratories) for 10 min in a humid chamber at room temperature in the dark. To avoid cross-reaction between the detection system for the first and second Abs, biotinylated goat anti-mouse IgM Ab was used at a lower concentration in the second step of the double-staining protocol, and controls were also performed omitting the second primary Ab (CD57) or replacing it with an isotype-matched control mAb with irrelevant specificity.

Statistical analysis

All data are presented as the mean ± SEM of duplicate or triplicate samples and represent at least three independent experiments. Analysis was performed using Student’s t test. A value of p < 0.05 was considered significant.

Results

CXCR4^+^ GC T cells migrate poorly to CXCL12

We previously reported that FDCs up-regulate CXCR4 on CD4 T cells and that a major population of GC CD4 T cells that bear CD57 and interact with FDCs in vivo also expresses high levels of CXCR4 (35). Because human T and B cells can migrate to FDCs (54), we hypothesized that CXCR4 may be involved in this process and sought to test this postulate. Because GC T cells bear up to 6-fold more CXCR4 than other CD4 T cells, we examined the migratory capacity of these cells to the CXCR4 ligand, CXCL12, and compared this to the migration of other CD4 T cells (CD57^-) from the same tissue. Remarkably, although GC T cells expressed >4-fold more CXCR4 than CD57^+ CD4 T cells from the same tissue (Fig. 1A), they migrated much less efficiently to CXCL12 than did the CD57^+ CD4 T cells (Fig. 1B). Because our GC T cell isolation procedure used positive selection for CD57, we excluded the possibility that anti-CD57 binding altered the migratory capacity of the cells to CXCL12. We negatively selected CD4 T cells and then phenotyped the cells after migration through the Transwell membrane to chemotaxtractant. These GC T cells were also nonresponsive to CXCL12 compared with CD57^+ CD4 T cells (data not shown).
FDCs express CXCL13, a potent chemoattractant for GC T cells

We next asked whether the nonresponsiveness of the GC T cells was specific to CXCL12 or whether they exhibited a generalized loss of migratory ability. Because GC T cells express CXCR5 (40), we analyzed their migration to the CXCR5 ligand, CXCL13. GC T cells expressed >4-fold higher surface expression of CXCR4 compared with the CD57-CD4 T cells. Purified CD57-CD4 T cells and GC T cells migrated to CXCL12 was determined after 5 h of culture in a Transwell migration system, after which the number of live cells in the lower chamber was counted. The data represent the mean ± SEM of triplicate wells. *, p < 0.01. The data are representative of six independent experiments.

Differential RGS gene expression by GC T cells correlates to impaired migration to CXCL12

The fact that GC T cells migrated efficiently toward CXCL13, but failed to migrate to CXCL12, suggested that these cells might be desensitized to signaling through CXCR4 even though their surface expression of this receptor was much higher than that of other CD4 T cells from the same tissue. To analyze the mechanism of
GC T cell nonresponsiveness to CXCL12, we compared the expression of several RGS genes in GC T cells and CD57+CD4 T cells. Because GC B cells highly express RGS1 and RGS13 and are also nonresponsive to CXCL12-mediated migration (50, 55), we reasoned that these genes may also be differentially expressed in GC T cells compared with CD57+CD4 T cells. We also analyzed the expression of two other genes expressed in T cells, RGS16 and RGS2. Interestingly, GC T cells expressed high levels of RGS13 mRNA (50) and observed that these lymphocytes also expressed RGS16 mRNA, albeit at ~6-fold lower levels than RGS13, whereas other B cells did not express detectable levels of either of these transcripts after 30 cycles of amplification (data not shown). To establish RGS13 and RGS16 protein expression in GC T cells, we obtained a previously

**FIGURE 3.** FDCs attract GC T cells via production of CXCL13. A, FDCs and FDC-depleted cells were purified and cultured in complete medium supplemented with 10% FBS for 6 days. Tissue culture supernatant from these cells was harvested, filtered through a 0.22-μm pore size filter, and used as the only source of chemoattractant in Transwell migration studies with purified GC T cells or CD57+ T cells. FDC supernatant attracted GC T cells, but not other CD4 T cells, whereas FDC-depleted supernatant did not attract any tonsillar CD4 T cells. B, FDC supernatant was pretreated with anti-CXCL13 (20 μg/ml) for 30 min at 37°C and used in Transwell migration assays with purified GC T cells. The addition of anti-CXCL13 reduced GC T cell migration by ~2-fold. C, GC T cells were pretreated with either anti-CXCR5 (10 μg) or control Ab (10 μg) for 30 min on ice before performing the migration studies with FDC supernatant. Addition of anti-CXCR5 abrogated migration to the FDC supernatant. The data represent the mean ± SEM of triplicate wells. *, p < 0.03. The data are representative of at least three independent experiments.

**FIGURE 4.** FDCs express CXCL13. A, mRNA was isolated from equal numbers of either MACS-enriched FDCs and FDC-depleted cells or FACS-purified FDCs and FDC-negative cells (cells not labeling with FDC-specific mAbs), followed by RT-PCR for β-actin and CXCL13. Southern blotting was performed using a labeled probe specific for CXCL13 and developed by ECL. Note the strong hybridization signal from FDCs, but not from FDC-depleted or negative cells. The data are representative of three independent experiments. B, CXCL13-specific sandwich ELISA was performed on five independent samples of supernatant fluid obtained from FDC cultures to determine the amount of CXCL13 produced. Culture of FDC-depleted cells did not detect the presence of CXCL13. The data represent the mean ± SEM of two or more replicates of 6-day cultured supernatant. *, p < 0.01.
characterized RGS13-specific Ab (provided by Dr. J. Kehrl, National Institute of Allergy and Infectious Diseases) (50) and generated a new anti-RGS16 Ab (RGS16 –210). Western blotting experiments confirmed the specificity of RGS16 –210 (Fig. 6A). Immunohistochemistry was then performed on tonsil sections to determine the expression of RGS13 and RGS16 (Fig. 6B) in vivo. RGS13 expression was largely confined to lymphoid follicles, and both GC T cells (CD57−) and other cells were labeled. Some interfollicular labeling was also observed. RGS16 expression was present in GC T cells and other follicular cells, but, in contrast to RGS13, was also found expressed in tonsillar epithelium.

Because GC T and B cells exist adjacent to FDCs in secondary lymphoid tissue and exhibit similar patterns of RGS13 and RGS16 gene expression, we hypothesized that FDCs could induce the expression of these genes in CD57− CD4 T cells, rendering them unresponsive to CXCL12. When CD57− CD4 T cells (which did not express RGS13 or RGS16) were cultured with FDCs for 24 h, both RGS13 and RGS16 mRNA were up-regulated (Fig. 7A); however, no changes were observed in their surface expression of CXCR5 or their ability to migrate to CXCL13 (data not shown). The expression of RGS13 and RGS16 was not up-regulated in short term cultures if the FDC and T cells were separated by a semipermeable Transwell membrane (Fig. 7A). Coculture of FDCs with CD57− CD4 T cells significantly impaired migration to CXCL12 (Fig. 7B). Thus, FDCs modulate RGS13 and RGS16 gene expression directly, regulating CD4 T cell migration to CXCL12. Therefore, GC T cells cultured in the absence of FDCs should down-regulate RGS13 and RGS16 to restore responsiveness to CXCL12. GC T cells cultured in the absence of FDCs for 24 h down-regulated both RGS13 and RGS16 mRNA (Fig. 8A), and this expression correlated directly with enhanced migration to CXCL12 (Fig. 8B). Furthermore, the specific migration of these GC T cells was equivalent to that of freshly isolated CD57− CD4 T cells. These data are consistent with the hypothesis that FDC-CDC-CD4 T cell interactions occur in vivo and that these interactions result in changes in RGS13 and RGS16 gene expression that result in GC T cell nonresponsiveness to CXCL12.

**Discussion**

Long term TD Ab responses, with high affinity IgG production, are critically dependent on a productive GC reaction, involving activated, Ag-specific B and T cells surrounding Ag-bearing FDCs (20, 27, 29, 56). Cellular localization of Ag-specific lymphocytes within lymphoid follicles is controlled by chemokines and the expression of chemokine receptors on these cells (1, 2). Because FDCs up-regulate CXCR4 on CD4 T cells, and GC T cells express high levels of CXCR4 (35), these cells should migrate efficiently to CXCL12. However, we observed that although GC T cells expressed high levels of CXCR4, they were nonresponsive to CXCL12. Such nonresponsiveness could be explained by increased expression of RGS13 and RGS16 in this cell population. When GC T cells were incubated in the absence of FDCs, they rapidly down-regulated CXCR4 and CXCL12 expression and became responsive to CXCL12. Additionally, incubation of CD57− CD4+ T cells with FDCs resulted in increased expression of RGS13 and RGS16, and this expression correlated with impaired CXCR4-evoked migration. Thus, the differential expression of RGS13 and RGS16 in GC T cells probably accounts for the observed CXCR4 desensitization. Our data also suggest that FDC contact with GC T cells in vivo results in inhibited migration to CXCL12 and that spatial separation from FDCs is required for restoration of CXCL12 responsiveness.

The nature of the FDC signal(s) responsible for up-regulation of RGS13 and RGS16 and impaired migration to CXCL12 is currently under investigation. Although the specific molecules involved in this signaling pathway have not yet been identified, our data suggest that FDC contact may be required to block migration to CXCL12, as was evidenced by the ablation of this effect when FDCs were separated from CD4 T cells by a Transwell in short term cultures. Although it is possible that our short term cultures did not allow the production of sufficient soluble signal to mediate RGS expression, Yuda et al. (34) found that CD57− GC T cells directly contact FDCs in secondary lymphoid tissue in vivo. The failure of GC T cells to migrate to CXCL12 is reminiscent of the inability of GC B cells to migrate to this chemokine despite high levels of CXCR4 expression (13). Interestingly, when GC B cells further differentiate, they regain responsiveness to CXCL12. This chemokine is expressed in areas surrounding GCs and in the bone marrow, and the differentiated B cells leave the GC by migrating toward CXCL12 (reviewed in Ref. 57). GC B cell nonresponsiveness to CXCL12 may be controlled by RGS1 and RGS13 expression (47, 50). In GC T cells, however, it is unlikely that RGS1 has a significant function in regulating CXCL12-induced migration,
because RGS1 mRNA was equivalent in migrating and nonmigrating CD4 T cell populations (Fig. 5). In contrast, CD57+CD4 T cells that migrated efficiently to CXCL12 expressed very low levels (often undetectable) of RGS13 and RGS16 mRNA, whereas GC T cells that were refractory to CXCL12 migration expressed high levels of these RGS genes (Fig. 5). Shi et al. (50) demonstrated RGS13 expression in cells located in the light zone of the GC, which is the site occupied by both GC B and T cells adjacent to FDCs. In addition, RGS13 strongly impairs signaling through Goi-regulated pathways, including those evoked by CXCR4 and CXCR5. Because GC T cells migrated efficiently to the CXCR5 ligand, CXCL13, RGS13 may preferentially inhibit CXCR4-mediated signaling. Although selectivity of RGS proteins for certain receptors has not been well established, some data suggest that this phenomenon may occur. RGS2, but not RGS4, binds directly to the third intracellular loop of specific muscarinic receptor subtypes (58). T cells from mice that transgenically overexpress RGS16...
exhibit impaired migration to CXCL12, but not CCL21 or CCL7 (59).

B cell differentiation signals may be important cues for egress of these cells from the GC to the bone marrow, where their differentiation into plasma cells is completed, and the bulk of Ab production occurs. Because it has been reported that B cell migration to CXCL12 is significantly reduced initially after BCR engagement (13), we hypothesize that GC B cell contact with Ag-bearing FDCs leads to the induction of CXCL12 nonresponsiveness. A subsequent signaling event must occur during the GC reaction that restores B cell migratory competence and allows them to leave the GC. Perhaps the release of immune complex-coated bodies (icososomes) from FDCs and their presentation to GC T cells by GC B cells (which can occur away from FDCs) result in the differentiation signals needed for plasma cell development (45, 60, 61) and the signals that restore migratory competence to CXCL12.

Regardless of whether GC B cell presentation of Ag to GC T cells also removes the block to GC T cell migration is unknown, but it is currently an area of active investigation. Our data indicate that removal of GC T cells from FDCs is required for restoration of migratory competence to CXCL12 because GC T cells cultured in the absence of FDCs resulted in decreased expression of RGS13 and RGS16 mRNA and regained responsiveness to CXCL12 when cultured in the absence of FDCs for 24 h. The data represent mRNA from one donor and are representative of two independent experiments. A, Freshly isolated GC T cells were incubated either at 4 or at 37°C in the absence of FDCs for 24 h, then assessed for their migratory ability to CXCL12 as described above. The data represent the mean ± SEM of triplicate wells. *, p < 0.01. The data are representative of at least two independent experiments. Note that CD57 CD4 T cell migration was reduced by 8-fold when cocultured with FDCs.

FIGURE 7. FDCs up-regulate the expression of RGS13 and RGS16 in CD57 CD4 T cells, which correlates with impaired CXCL12 migration. A, Purified CD57 CD4 T cells were cultured alone, with FDCs, or separated from FDCs by a 0.4-μm pore size Transwell filter. The FDC control represents RNA isolated from CD57 T cells cultured alone for 24 h pooled with FDCs cultured alone for this same period of time. This control indicates that FDCs were not the source of the expressed RGS mRNA in the coculture experiment. The data represent mRNA expression from one donor and are representative of three independent experiments. B, Purified CD57 CD4 T cells were cultured alone or with FDCs for 24 h, and then 5 × 10⁵ cells were placed in a Transwell migration assay as described above using CXCL12 as chemoattractant. The FDC control consists of FDCs and CD4 CD57 T cells cultured separately and then combined immediately before the migration assay. This control ensures that T cell migration to CXCL12 is not impaired by FDC production of CXCL13 or physical inhibition of T cell migration. The data represent the mean ± SEM of triplicate wells. *, p < 0.0001. The data are representative of at least two independent experiments. Note that CD57 CD4 T cell migration was reduced by 8-fold when cocultured with FDCs.

FIGURE 8. GC T cells down-regulated RGS13 and RGS16 mRNA and regained responsiveness to CXCL12 when cultured in the absence of FDCs. A, RNA was isolated from freshly isolated, purified GC T cells or from GC T cells cultured in the absence of FDCs for 24 h. The data represent mRNA from one donor and are representative of two independent experiments. B, Freshly isolated GC T cells were incubated either at 4 or at 37°C in the absence of FDCs for 24 h, then assessed for their migratory ability to CXCL12 as described above. The data represent the mean ± SEM of triplicate wells. *, p < 0.01. The data are representative of at least two independent experiments.
also apparent in human secondary lymphoid tissues, suggesting that FDCs (which are organized in a reticulum) are the major producers of CXCL13 (43, 49, 62). Thus, FDCs appear to not only provide Ag for maintenance of long term Ab responses, but also to secrete a chemokine, CXCL13, that attracts Ag-specific B and T cells into the lymphoid follicle to initiate the GC reaction and retain them in this location (through CXCL12 nonresponsiveness).

It seems paradoxical that FDCs up-regulate CXCR4 expression in GC T lymphocytes (35), yet induce RGS expression to inhibit CXCR4-evoked signaling. We reason that regulation of CXCL12 responsiveness during the first phase of the GC reaction may serve to keep Ag-specific B and T cells within GCs to induce Ab-forming cell generation (45, 57). We envision that FDCs secrete CXCL13 to attract Ag-specific B and T cells from the site of initial activation by dendritic cells in the TD zones of secondary lymphoid tissues. Although the frequency of Ag-specific B and T cells is low, GC development requires both cell types to be present. Premature migration would probably result in the inability of the GC reaction to progress, resulting in an inefficient response to TD Ags. Therefore, up-regulation of CXCR4, followed by down-regulation of its signaling capacity (until additional signals are provided), would allow Ag-specific cells to be retained in the GC for sufficient time periods to induce the generation of Ab-forming cells. Thereafter, when the majority of memory B cells are generated, the nature and type of T cell signals would be altered.

In addition to contributing to the GC reaction, another potentially important consequence of FDC inhibition of GC T cell migration out of the follicle relates to HIV pathogenesis. FDCs are a long term repository of infectious virus trapped early in HIV infection (46, 63–65). FDC inhibition of GC T cell migration may be an initially important consequence of FDC inhibition of GC T cell migration from the T cell areas of lymphoid organs. J. Exp. Med. 189:447.


