B Lymphocyte Chemotaxis Regulated in Association with Microanatomic Localization, Differentiation State, and B Cell Receptor Engagement

By Conrad C. Bleul,* Joachim L. Schultze,† and Timothy A. Springer*

From *The Center for Blood Research and Harvard Medical School, Department of Pathology, Boston, Massachusetts 02115; and the †Department of Adult Oncology, Dana-Farber Cancer Institute and Harvard Medical School, Boston, MA 02115

Summary

Migration of mature B lymphocytes within secondary lymphoid organs and recirculation between these sites are thought to allow B cells to obtain T cell help, to undergo somatic hypermutation, to differentiate into effector cells, and to home to sites of antibody production. The mechanisms that direct migration of B lymphocytes are unknown, but there is evidence that G protein-coupled receptors, and possibly chemokine receptors, may be involved. Stromal cell-derived factor (SDF)-1α is a CXC chemokine previously characterized as an efficacious chemoattractant for T lymphocytes and monocytes in peripheral blood. Here we show with purified tonsillar B cells that SDF-1α also attracts naive and memory, but not germinal center (GC) B lymphocytes. Furthermore, GC B cells could be converted to respond to SDF-1α by in vitro differentiation into memory B lymphocytes. Conversely, the migratory response in naive and memory B cells was significantly reduced after B cell receptor engagement and CD40 signaling. The receptor for SDF-1, CXC chemokine receptor 4 (CXCR4), was found to be expressed on responsive as well as unresponsive B cell subsets, but was more rapidly downregulated on responsive cells by ligand. Finally, messenger RNA for SDF-1 was detected by in situ hybridization in a layer of cells surrounding the GC. These findings show that responsiveness to the chemoattractant SDF-1α is regulated during B lymphocyte activation, and correlates with positioning of B lymphocytes within a secondary lymphoid organ.

Mature B lymphocytes are known to localize to distinct microenvironments within secondary lymphoid organs such as the spleen, peripheral and mesenteric lymph nodes, and Peyer’s patches in the gut. In the spleen, these microenvironments are located in the white pulp and support antigen- and costimulus-dependent proliferation in the T cell zone (periarteriolar lymphocyte sheath, PALS) and affinity maturation in the germinal center (GC) within the B cell area or follicle. Corresponding compartments can be found in the other secondary lymphoid organs. Early studies in the rat have shown that recirculating B cells collected from the thoracic duct home to the follicular mantle zone, i.e., the B cell area surrounding GCs, but not to the GC itself (1, 2). A large body of work on the generation of T cell-dependent antibody responses suggests that B lymphocytes after encounter of antigen and B cell receptor (BCR) engagement are initially activated by cognate interaction with T lymphocytes in the T cell zone (3–5). B cells then move to the follicle where they form germinal centers in which they undergo somatic hypermutation and affinity maturation. After the GC reaction wanes, some memory B cells are thought to remain in close proximity to follicular dendritic cells within the B cell area, whereas others recirculate or migrate together with antibody-secreting cells into the bone marrow (6).

The regulated movement of B lymphocytes into supportive niches is thought to prevent autoreactivity. Goodnow and coworkers have demonstrated that in the presence of a polyclonal B cell repertoire, a process termed follicular exclusion hinders autoreactive B cells from entering the B cell zone, which is a prerequisite for their survival in the absence of T cell help (7–9). Follicular exclusion has been proposed to be due to subtle differences between autoreactive and nonautoreactive B cells in responsiveness to positioning cues and is thought to be a checkpoint for censoring autoreactive B cells from the preimmune repertoire (9). This concept has recently been challenged by studies showing that BCR engagement and not the presence or absence

Abbreviations used in this paper: BCR, B cell receptor; BLR1, Burkitt’s lymphoma receptor 1; DIG, digoxigenin; F-actin, filamentous actin; GC, germinal center; IP10, interferon-inducible protein 10; L, ligand; Mig, monokine-induced by IFN-γ; MIP, macrophage inflammatory protein; mRNA, messenger RNA; SDF, stromal cell-derived factor; SSC, standard saline citrate; t, transfected.
of a polyclonal B cell repertoire is the key factor that leads to arrest of B cells in the outer periarteriolar lymphoid sheath (10, 11).

The molecular mechanisms that govern localization of B cells within secondary lymphoid organs and between these sites are unknown, but some studies suggest the involvement of seven transmembrane receptors and signaling through pertussis-toxin-sensitive G proteins. Goodnow's group has shown in transfer experiments that pretreatment with pertussis-toxin inhibits the migration of B and T cells into the splenic white pulp indicating that a G protein-coupled receptor, and perhaps one that binds a chemotactic factor, may be necessary for attraction of lymphocytes into the white pulp (12). A recent study provides evidence for a role of the putative chemokine receptor Burkitt's lymphoma receptor 1 (BLR 1) in B cell migration. The phenotype of BLR 1-deficient mice suggests the importance of this receptor in the localization of B lymphocytes within follicles in the spleen (13).

The role of chemotactic factors in directing granulocytes, macrophages, and T lymphocytes from the bloodstream into sites of inflammation has been extensively investigated (14, 15), but very little is known about B lymphocyte chemoattractants and their receptors. To characterize chemoattractant-induced B cell migration, we carried out chemotaxis assays with stromal cell-derived factor (SDF)-1α, which we have shown to be an efficacious chemoattractant for monocytes and lymphocytes, including the CD4+ and CD8+ T lymphocyte subsets, and the CD45R A+ naïve and CD45R O- memory T lymphocyte subsets in peripheral blood (16). SDF-1 is unique among chemotactic cytokines. Formally, SDF-1 belongs to the CXC subfamily of a family of small, 60–80 amino acid chemotactic cytokines termed chemokines. All other human CXC chemokine genes cluster on chromosome 4; the sdf-1 gene is located on chromosome 10 (17). SDF-1 is with 99% identity between mouse and human the most highly conserved cytokine described to date (16, 17). Mice genetically deficient in SDF-1 lack B lymphocytes, lack myelopoiesis in the bone marrow, and show defects in heart development (18). Such a severe phenotype has not been described for any other chemokine and demonstrates wide importance for SDF-1 in both hematopoietic and nonhematopoietic organs. The defect in B cell development is in agreement with the finding that SDF-1 functions as a pre-B cell growth-stimulating factor (19).

It has recently been shown that SDF-1α binds the CXC chemokine receptor 4 (CXCR 4; references 20, 21). CXCR 4, also known as LEST R/fusin, facilitates entry of T cell lineotropic strains of HIV-1 into CD4-expressing cells (22). By binding to CXCR 4, SDF-1α blocks infection by these strains of HIV-1 (20, 21). Expression of CXCR 4 messenger RNA (mRNA) has been detected in a wide variety of tissues (23–25) and we have found expression of CXCR 4 on T and B lymphocytes on the protein level (26).

B lymphocytes differ from T lymphocytes in that they can undergo a second round of antigen receptor editing. During development, both T and B lymphocytes go through VDJ recombination in a primary lymphoid organ. However, after encounter of antigen, B lymphocytes undergo somatic hypermutation, and also further recombination, in a specialized microenvironment in secondary lymphoid organs, the GC. The surface phenotype of tonsillar B cells is characteristic for the localization in the different compartments of the human tonsil and have been well characterized. Here, we investigate the responsiveness to SDF-1α of tonsillar B lymphocytes with distinct surface phenotypes corresponding to different microenvironments within this secondary lymphoid organ.

Materials and Methods

Cytokines, Chemokines, and Antibodies. IL-2 was a kind gift of Dr. J. Ritz (Dana Farber Cancer Institute, Boston, MA) and IL-10 was purchased from Genzyme (Cambridge, MA). Synthetic, human SDF-1α (1–67) (20) was a gift of Dr. I. Clark-Lewis (University of British Columbia, Vancouver, Canada). IL-8, macrophage inflammatory protein (MIP)-1α, MIP-1β, monocyte chemotactic protein (MCP)-1, and regulated on activation normal T cell expressed and secreted (RANTES) were obtained from Genzyme, monokine induced by IFN-γ (Mig) from Pharmingen (San Diego, CA), interferon-inducible protein 10 (IP10) from R&D Sys. Inc. (Minneapolis, MN), and lymphotactin from Peprotec (Rocky Hill, NJ). The following mAbs and polyclonal antibodies were used for staining of surface antigens: anti-kappa-FITC was obtained from Dako (Carpinteria, CA); anti-intercellular adhesion molecule (ICAM)-1–PE, anti-CD20-FITC and -PerCP, anti-CD3-PerCP, anti-CD14-PE, and anti-CD38-PE from Beckman Coulter (Brea, CA); anti–B7.1 (CD80) was a gift of Dr. C.R. Mackay (LeukoSite Inc., Cambridge, MA), anti-B7.2 (CD86) from PharMingen; anti-B7.3 (CD83) from PharMingen; anti-CD44-PE, anti-CD56-PE, anti-CD62 ligand (L)-FITC, anti-CD107a–PE, and anti-CD25–PE from Beckman Coulter (Brea, CA). The following mAbs and polyclonal antibodies were used for surface staining: anti-CD2–FITC, anti-CD3–FITC, anti-CD5–FITC, anti-CD7–FITC, anti-CD19–FITC, anti-CD22–FITC, anti-CD4-PerCP, anti-CD8–PerCP, anti-CD16–PerCP, anti-CD19–PerCP, anti-CD22–PerCP, anti-CD3–PerCP, anti-CD4–PerCP, anti-CD45–PerCP, anti-CD64–PerCP, anti-CD85–PerCP from eBioscience (San Diego, CA), interferon-inducible protein 10 (IP10) from R&D Sys. Inc. (Minneapolis, MN), and lymphotactin from Peprotec (Rocky Hill, NJ). The following mAbs and polyclonal antibodies were used for staining of surface antigens: anti-kappa-FITC was obtained from Dako (Carpinteria, CA); anti-intercellular adhesion molecule (ICAM)-1–PE, anti-CD20-FITC and -PerCP, anti-CD3-PerCP, anti-CD14-PE, and anti-CD38-PE from Beckman Coulter (Brea, CA); anti–B7.1 (CD80) was a gift of Dr. C.R. Mackay (LeukoSite Inc., Cambridge, MA), and the mAb 12G5 against CXCR4 (28) was supplied by Dr. J. Hoxie (University of Pennsylvania, Philadelphia, PA). Unconjugated mAbs directed against CD38, CD44, kappa, and lambda light chain were obtained from Immunotech (Westbrook, ME); anti-CXCR4-PE (12G5) from Pharmingen; anti-B7.1 (CD80) was obtained from R&D Systems (Minneapolis, MN), and B7.2 (CD86) from Pharmingen (San Diego, CA). The annexin V apoptosis detection assay was carried out according to the manufacturer's recommendations (R&D Sys. Inc.).

B Lymphocyte Purification. Fresh tonsils were obtained from the Massachusetts Eye and Ear Infirmary (Boston, MA). Tonsils were mechanically homogenized. Tonsillar B lymphocytes were obtained from the single cell suspension by rosetting with sheep red blood cells (BioW hittaker, Walkersville, MD). The purity of tonsillar B cells obtained with this method was routinely >90% as estimated by FACScan® analysis (Beckton Dickinson, San Jose, CA) using mAb to CD20, CD19, CD3, CD4, CD8, and CD14. Subsets of tonsillar B cells were purified by negative selection using a magnetic cell separation protocol (MACS; Miltenyi Biotec, Sunnyvale, CA). In brief, tonsillar B cells were incubated with mAbs specific for CD3, CD14, and either CD20 (for naïve and memory B cell preparation), or CD44 (for GC B cell preparation), and subsequently with magnetic beads coated with anti–mouse IgG antibodies. Unstained cells were separated on a MACS col-

754 B Lymphocyte Chemotaxis Regulated by Differentiation and BCR Engagement
In brief, 5 m migrants for a period of 4 h and B cell subsets for a period of 3 h.

In vitro generation of memory B cells. Purified GC B cells were transferred into memory type B cells in vitro as described (30). In brief, human B cell subsets (1.25 × 10^6/ml) were incubated in L15 medium at 37°C in RPMI-1640 containing 2% human AB serum, 1% human serum albumin, 50 μg/ml gentamicin (B cell medium) and the mean relative fluorescence of each sample determined.

R E S U L T S

Chemotaxis. To characterize chemotactic responses in B lymphocytes we used purified human tonsillar B cells in a chemotaxis assay with bare polycarbonate filters with 5-μm pores as part of a transwell insert (16, 33). In contrast to the Boyden chamber microchemotaxis assay, this assay allows the determination of the absolute number of responsive cells and the collection and characterization of transmigrated cells. Tonsillar B lymphocytes were chosen because they comprise subpopulations in multiple states of activation and differentiation that have been well characterized according to their surface antigen expression and localization in tonsil (34–37). Using the bare filter chemotaxis assay, we detected specific migration of a small fraction of tonsillar B cells towards SDF-1α, which peaked at a concentration of 1.5 μg/ml of the chemokine and showed the typical biphasic curve that is characteristic for chemoattractant induced migration (Fig. 1A, top). The same concentration was found to be optimal for T lymphocyte chemotaxis (16). Depending on the donor, the percentage of responsive cells varied in nine independent experiments between 6 and 20% of total input (data not available).
expected. It also is possible that SDF-1α may use additional, as yet unidentified, receptors on B lymphocytes.

The CXC chemokines IL-8, MIP, and IP10, the CC chemokines MIP-1α, MIP-1β, monocyte chemotactic protein 1, and regulated on activation normal T cell expressed and secreted (RANTES), the C chemokine lymphotactic, and the chemotactic C5a were also tested for their ability to induce chemotaxis in tonsillar B lymphocytes over a 1,000-fold concentration range in the same assay. None of the tested chemokines induced significant migration in at least two independent experiments. Although a low amount of activity was seen with MIP and IP10 in some experiments, this was not a consistent finding (data not shown).

Characterization of SDF-1α Responsive B Lymphocyte Subsets. We collected and phenotyped transmigrated tonsillar B cells. Three main subpopulations have been described (3, 34–39). GC B cells express CD38, high levels of CD20, and little to no CD44 and surface immunoglobulin. B cells outside the GC are negative for CD38, and express CD44 and intermediate levels of CD20. B cells outside the GC can be divided into CD38−CD44−IgD+ naïve and CD38+CD44+IgD− memory B lymphocytes. The input population of purified tonsillar B cells contained about two-fold more CD38+GC B cells than CD38−GC B cells (Fig. 1 A, upper left). By contrast, almost all transmigrated cells were negative for CD38 (Fig. 1 B, upper right). Among transmigrated B lymphocytes, half were CD38−IgD+ naïve and the other half were CD38+IgD− memory B cells (Fig. 1 B, lower right). Both subpopulations were present in the input population in a similar 1:1 ratio (Fig. 1 B, lower left). CD38+GC B cells were consistently unresponsive in 11 independent experiments.

GC B cells are large, blast-like lymphocytes that are thought to undergo somatic hypermutation within the specialized microenvironment of the GC. It is unlikely, though, that the unresponsiveness to SDF-1α is due to the increased size of GC B lymphocytes, since phytohemagglutinin-stimulated T lymphocyte blasts that are even larger in size than GC B cells as estimated by forward light scatter readily respond to SDF-1α in the same assay (26). It has been shown that isolated GC B cells rapidly undergo apoptosis (40). To monitor apoptosis among input tonsillar B lymphocytes, we tested input cells at different time points over the 4-h duration of the chemotaxis assay for binding of annexin V. Loss of membrane phospholipid asymmetry, which leads to surface binding of annexin V, is thought to be an early marker of programmed cell death (41). We found that after 2 h, ~20% and after 4 h, ~30% of input cells bound annexin V, indicating that the majority of input GC cells remained intact during the course of the assay.

To confirm the unresponsiveness of GC B lymphocytes to SDF-1α, we carried out chemotaxis assays with isolated B cell subsets. Purified CD38+GC B cells showed little, if any, specific migration above background (Fig. 2 A), whereas 50–80% of isolated CD38− naïve and memory B lymphocytes migrated to an optimal concentration of SDF-1α of 1.5 µg/ml in the same assay (Fig. 2 A). Specific migra-
cells within 15 s after addition of SDF-1α in purified CD38

Differentiation State and Chemotaxis. The finding that the differentiation state of a B lymphocyte correlates with its responsiveness to chemokine prompted us to test if in vitro differentiation of isolated cells would restore migratory responses in the same cells. To this end we purified GC B cells and differentiated them into memory type B lymphocytes for 2 h with anti-kappa and -lambda light chain mAbs in the presence of NIH3T3 cells that express CD38 (to obtain naive and memory B cells) or CD44 (to obtain GC B cells) and tested in the chemotaxis assay to an optimal concentration of SDF-1α of 1.5 μg/ml. Results are shown as specific migration, i.e., migration above background migration to medium alone, and represent mean and standard deviation of three independent experiments.

Figure 2. SDF-1α efficiently attracts purified CD38− naive and memory B lymphocytes by a pertussis toxin-sensitive mechanism. (A) B lymphocyte subsets were isolated by negative selection with an mAb specific for CD38 (to obtain naive and memory B cells) or CD44 (to obtain GC B cells) and tested in the chemotaxis assay to an optimal concentration of SDF-1α of 1.5 μg/ml. Results are shown as specific migration, i.e., migration above background migration to medium alone, and represent mean and standard deviation of three independent experiments. (B) Isolated CD38− naive and memory B lymphocytes were transmigrated to an optimal concentration of SDF-1α after pretreatment with or without pertussis toxin (PT) over 2 h at 37°C. Control shows migration of sham treated cells to buffer alone. Columns and error bars show the mean and standard deviation of three independent experiments.

The presented experiments suggest that B lymphocytes participating in the GC reaction are unresponsive to chemotactic stimulation by SDF-1α. As a second, independent assay that determines responsiveness to SDF-1α, we carried out actin polymerization experiments with CD38− GC B cells versus CD38+ B cells. In this assay, changes in intracellular filamentous actin (F-actin) were measured after exposure to SDF-1α over time (29). There was a significant, transient increase in intracellular F-actin in purified CD38− naive and memory B cells within 15 s after addition of SDF-1α, whereas F-actin in purified CD38+ GC B cells showed little change compared to sham-treated cells (Fig. 3). The observed rapid increase in filamentous actin is characteristic for chemokine induced actin polymerization and differs from stimulation with phorbol esters and calcium ionophores (29, 42, 43).

The presented experiments suggest that B lymphocytes participating in the GC reaction are unresponsive to chemotactic stimulation by SDF-1α.

Figure 3. Naive and memory, but not GC B lymphocytes respond to SDF-1α by reorganization of the actin skeleton. Intracellular F-actin was measured using FITC-labeled phalloidin in purified CD38− naive and memory B cells (boxes) and CD38+ GC B cells (circles) after addition of SDF-1α at time 0 at a concentration of 1.5 μg/ml or in untreated CD38− naive and memory B cells (diamonds). Results are shown as percent of intracellular F-actin relative to the value before addition of SDF-1α and are the mean and standard deviation of three independent experiments.
This treatment reduced SDF-1α-mediated chemotaxis consistently by 50% (Fig. 5A). Treatment for longer than 2 h did not result in additional reduction in migration indicating that BCR cross-linking regulates responsiveness to SDF-1α within hours. Adding an mAb specific for CD40 to the combination of anti-kappa and -lambda light chain mAbs under the same conditions led to an additional reduction in SDF-1α-induced migration (Fig. 5A). In contrast, the anti-CD40 mAb alone had no effect on B cell chemotaxis to SDF-1α (Fig. 5A). To demonstrate the specificity of the inhibitory effect of BCR cross-linking on SDF-1α-induced migration, we incubated CD38− naive and memory B cells for 2 h in the presence of NIH3T3 cells expressing FcyRII with a lambda light chain–specific mAb alone. After chemotaxis to an optimal concentration of SDF-1α, input and transmigrated cells were stained for kappa light chain expression (Fig. 5B). BCR cross-linking with an mAb specific for lambda light chain consistently led to a selective reduction in the percentage of lambda-expressing B lymphocytes in the transmigrated population compared to the input population, as shown by the increase in the percentage of transmigrated B cells that expressed kappa chain.

Differential Downregulation of CXCR4 by SDF-1α. Chemokine directed migration is mediated through G pro-
To investigate if the unresponsiveness of GC B lymphocytes was due to a lack of expression of CXCR4, the receptor for SDF-1, we stained tonsillar B lymphocytes containing responsive and unresponsive subsets with the CXCR4-specific mAb 12G5. We found that both subsets express high levels of the receptor (Fig. 6A). Exposure to SDF-1 at 37°C downregulated surface expression of CXCR4. Interestingly, receptors on CD38 naive and memory B cells were more rapidly downregulated than on CD38 GC B cells in multiple experiments, with a 73 and 54% decrease, respectively, after 30 min (Fig. 6B). The same treatment with SDF-1 carried out at 4°C did not result in diminished binding of mAb 12G5 (not shown). This finding indicates that the reduced binding of CXCR4-specific mAb after treatment at 37°C is due to true downregulation of the receptor and not competition of the antibody and the ligand for the same epitope.

Expression of SDF-1 production within the human tonsil, we carried out in situ hybridization studies on frozen sections of human tonsil. Using a RNA probe specific for both SDF-1α and SDF-1β we detected message for SDF-1 in cells present in the connective tissue that forms the stationary scaffold of the tonsil and in non-lymphoid cells that surround GCs (Fig. 7). Expression of SDF-1 was found to be excluded from GCs and not to be associated with vascular or epithelial components in the tonsillar tissue. The staining found in the tonsil therefore suggests expression of SDF-1 in a specialized reticulum cell that lines the GC.

Discussion

SDF-1α induces migration in naive and memory B cells, whereas GC B cells are unresponsive in assays measuring chemotaxis and actin polymerization. B lymphocytes within the GC maintain high levels of the SDF-1 receptor CXCR4. Despite the expression of the appropriate chemokine receptor on these cells, there is no ligand-induced chemotaxis. Our studies further indicate that in comparison to naive and memory B cells, on GC B cells CXCR4 is more slowly removed from the surface after addition of SDF-1α. Internalization of chemokine receptors within minutes after addition of agonist, as shown here for CXCR4 on naive and memory B lymphocytes after addition of SDF-1α, has been described for the FMLP and C5a receptors and is thought to be an important mechanism to allow the continuous sampling of ambient chemotactic concentration that is necessary to follow a chemotactic gradient (46-48).
It seems that in GC B cells, CXCR4 is less coupled to downstream regulators that induce internalization of receptors than in migratory cells; there may be overlap between these downstream regulators and those that signal chemotaxis.

The function of CXCR4 on GC B cells remains enigmatic. SDF-1 has previously been described to promote growth of pre-B cells and has therefore been termed PBSF for pre-B cell growth-stimulating factor (19). We have carried out preliminary experiments measuring apoptosis in isolated GC B lymphocytes in the presence or absence of SDF-1α and have failed to demonstrate a significant effect on inhibition of apoptosis in these cells. In addition, our in situ hybridization experiments indicate that expression of SDF-1 is excluded from GCs. Thus, we have no indication that SDF-1 serves as a growth factor or survival signal to GC B cells that express its specific receptor CXCR4. It has to be kept in mind that additional not yet identified factors may use CXCR4 on B cells within GCs.

SDF-1α induces migration in naive and memory B cells, but not in GC B cells. This observation suggests that B lymphocytes undergoing somatic hypermutation within GCs are unresponsive to cues that direct migration of naive and memory B lymphocytes. In the mouse, Butcher’s group has shown that purified PNAhi GC B cells compared to B cells outside of the GC do not home to secondary lymphoid organs when transferred into syngeneic recipients (49). Although the lack of expression of the homing receptor L-selectin is thought to be a factor, other mechanisms are likely to be involved since homing to the spleen, which is L-selectin independent (50), was also impaired (49). The authors have proposed that the nonmigratory phase is a transient phenomenon that is related to a specific differentiation state within the confines of the GC microenvironment and our observation that in vitro differentiation of GC B cells toward a memory phenotype restores responsiveness to SDF-1 supports this notion.

Responsiveness of B lymphocytes to SDF-1α is regulated by differentiation state and BCR signaling. In contrast to the transition from unresponsive GC B cells to migratory effector B lymphocytes that developed over days in culture, the responsiveness to SDF-1α in CD38+ naive and memory cells decreased rapidly after BCR signaling. Only hours after BCR cross-linking, naive and memory B cells migrated significantly slower than control cells, and this effect was increased by concomitant signaling through CD40. CD40 signaling by itself was ineffective. Thus, while responsiveness of naive and memory B lymphocytes to SDF-1α is reduced after BCR cross-linking, these cells are thought to migrate to the T cell areas of secondary lymphoid organs after encounter of antigen (5, 51). This finding should not be interpreted as contradictory to our results since SDF-1 is unlikely to be the only chemotactic factor acting on B lymphocytes in lymphoid organs. Our finding may therefore suggest that the reduced responsiveness to SDF-1 allows a B cell after encounter of antigen to migrate along a gradient of a different chemotactic factor leading it into the T cell area. In this context, our observation that SDF-1 mRNA is found in a specialized reticulum cell that lines GCs in the tonsil is particularly striking. The expression of mRNA for SDF-1 in nonlymphoid cells surrounding GCs correlates with the position of small recirculating naive and memory B lymphocytes in the tonsil (38). Our finding is consistent with the previous isolation of SDF-1 protein or mRNA from stromal cell lines, in this case derived from the bone marrow (16, 19, 52). Our in situ hybridization results, therefore, further support the finding that responsiveness to SDF-1 correlates with the localization of B cell subsets within the tonsil.

Much is known about the events that allow emigration of lymphocytes from the bloodstream, through high endothelial venules into secondary lymphoid organs (14, 15), whereas little is known about the subsequent steps that direct migration of B and T lymphocytes into their respective microenvironments. Our study shows that subsets of B lymphocytes within a secondary lymphoid organ respond to SDF-1α and that this responsiveness correlates with a specific localization within this lymphoid organ and is regulated by the differentiation state of the cell and by BCR engagement. Migration of pre- and pre-B cells to SDF-1α has also recently been described in the mouse (53). Our previous work demonstrated that naive and memory T lymphocytes in the peripheral blood efficiently migrate towards an SDF-1α gradient (16). Chemokines may be important to direct migration of lymphocytes into supportive microenvironments and to maintain the specialized architecture of secondary lymphoid organs. Our work suggests that SDF-1α may participate in this process, but further work will be necessary to investigate the importance of SDF-1 relative to other chemokines. The unresponsiveness of GC B cells to SDF-1 may be due to alterations in downstream signaling pathways, and thus this may generalize to other chemotactant receptors. From work on the putative chemokine receptor BLR1, it becomes clear that distinct ligand–receptor pairs may be responsible for the positioning of B cells in the spleen and Peyer’s patches versus mesenteric and peripheral lymph nodes, since GC architecture is compromised in BLR1-deficient mice only in the spleen and Peyer’s patches (13). The situation is complicated by the fact that lymphocytes express multiple relevant chemokine receptors that allow detection of multiple concentration gradients, and they may influence each other within a given microenvironment. Further work will be necessary to identify other B lymphocyte chemotactants to address these questions.

We are indebted to Sabine Michalak for excellent technical support. The authors thank Qing Ma for help with RNA probes and in situ hybridization and Cheryl Greene at the Massachusetts Eye and Ear Infirmary (Boston, MA) for supplying human tonsils.
References

1. Nieuwenhuis, P., and W.L. Ford. 1976. Comparative migration of B- and T-lymphocytes in the rat spleen and lymph nodes. Cell. Immunol. 23:254–267.

2. Howard, J.C., S.V. Hunt, and J.L. Gowans. 1972. Identification of marrow-derived small lymphocytes in the lymphoid tissue and thoracic duct lymph of normal rats. J. Exp. Med. 135:200–219.

3. MacLennan, I.C.M. 1994. Germinal centers. Annu. Rev. Immunol. 12:117–139.

4. Rajewsky, K. 1996. Clonal selection and learning in the antibody system. Nature. 381:751–758.

5. Kelsoe, G. 1996. Life and death in germinal centers (Redux). Nature. 382:635–638.

6. Paramithiotis, E., and M.D. Cooper. 1997. Memory B lymphocytes migrate to bone marrow in humans. Proc. Natl. Acad. Sci. USA. 94:208–212.

7. Cyster, J.G., S.B. Hartley, and C.C. Goodnow. 1994. Competition for follicular niches excludes self-reactive cells from the recirculating B-cell repertoire. Nature. 371:389–395.

8. Cyster, J.G., and C.C. Goodnow. 1995. Antigen-induced exclusion from follicles and anergy are separate and complementary processes that influence peripheral B cell fate. Immunol. 3:691–701.

9. Goodnow, C.C., J.G. Cyster, S.B. Hartley, S.E. Bell, M.P. Cooke, J.L. Healy, S. Akkaraju, J.C. Rathmell, S.L. Pogue, and K.P. Shokat. 1995. Self-tolerance checkpoints in B lymphocytes. Annu. Rev. Immunol. 13:350–381.

10. Fulcher, D.A., A.B. Lyons, S.L. Korn, M.C. Cook, C. Koleda, C. Parish, B. Fazekas de St.Groth, and A. Basten. 1996. The fate of self-reactive B cells depends primarily on the degree of antigen receptor engagement and availability of T cell help. J. Exp. Med. 183:2313–2328.

11. Cook, M.C., A. Basten, and B. Fazekas de St.Groth. 1997. Ovarian peritubular lymphoid sheath arrest and subsequent differentiation of both naive and tolerant immunoglobulin transgenic B cells is determined by B cell receptor occupancy. J. Exp. Med. 186:631–643.

12. Cyster, J.G., and C.C. Goodnow. 1995. Pertussis toxin inhibits migration of B and T lymphocytes into splenic white pulp cords. J. Exp. Med. 182:581–586.

13. Forster, R., A.E. Matis, E. Kremmer, E. Wölf, G. Bren, and M. Lipp. 1996. A putative chemokine receptor, BLR1, directs B cell migration to defined lymphoid organs and specific anatomic compartments of the spleen. Cell. 87:1037–1047.

14. Springer, T.A. 1995. Traffic signals on endothelium for lymphocyte recirculation and leukocyte emigration. Annu. Rev. Physiol. 57:827–872.

15. Butcher, E.C., and L.J. Picker. 1996. Lymphocyte homing and homeostasis. Science. 272:60–66.

16. Bleul, C.C., R.C. Fuhlbirger, J.M. Casenova, A. Aiuti, and T.A. Springer. 1996. A highly efficacious lymphocyte chemoattractant, stromal cell-derived factor 1 (SDF-1). J. Exp. Med. 184:1101–1110.

17. Shirouzu, M., T. Nakano, J. Inazawa, K. Tashiro, H. Tada, T. Shinohara, and T. Honjo. 1995. Structure and chromosomal localization of the human stromal cell-derived factor 1 (SDF1) gene. Genomics. 28:495–500.

18. Nagasawa, T., S. Hirota, K. Tachibana, S.-I. Nishikawa, Y. Kitamura, N. Yoshida, H. Kikutani, and T. Kishimoto. 1996. Defects of B-cell lymphopoiesis and bone marrow myelopoiesis in mice lacking the CXC chemokine PBSF/SDF-1. Nature. 382:635–638.

19. Nagasawa, T., H. Kikutani, and T. Kishimoto. 1994. Molecular cloning and structure of a pre-B-cell growth-stimulating factor. Proc. Natl. Acad. Sci. USA. 91:2305–2309.

20. Bleul, C.C., M. Farzan, H. Choe, C. Parolin, I. Clark-Lewis, J. Sodroski, and T.A. Springer. 1996. The lymphocyte chemoattractant SDF-1 is a ligand for LESTR/fusin and blocks HIV-1 entry. Nature. 382:829–833.

21. Oberlin, E., A. Amara, F. Bacherie, C. Besia, J.-L. Virelizier, A. Arenalal-Seisdedos, O. Schwartz, J.-M. Heard, I. Clark-Lewis, D.F. Legler, et al. 1996. The CXC chemokine, stromal cell derived factor 1 (SDF-1), is the ligand for LESTR/fusin and prevents infection by T-cell-line-adapted HIV-1. Nature. 382:833–835.

22. Feng, Y., C.C. Broder, P.E. Kennedy, and E.A. Berger. 1996. HIV-1 entry cofactor: functional cDNA cloning of a seven-transmembrane, G protein-coupled receptor. Science. 272:877–882.

23. Federspiel, B., I.G. Melhado, A.M. Duncan, A. Delaney, K. Schappert, I. Clark-Lewis, and F.R. Jirik. 1993. Molecular cloning of the cDNA and chromosomal localization of the gene for a putative seven-transmembrane segment (7-TMS) receptor isolated from human spleen. Genomics. 16:707–712.

24. Noma, H., B.W. Nielsen, and M. Matsushima. 1993. Molecular cloning of cDNAs encoding a LD78 receptor and putative leukocyte chemotactic peptide receptors. Int. Immunol. 5:1239–1249.

25. Loetscher, M., T. Geiser, T. O'Reilly, R. Zwahlen, M. Baggiolini, and B. Moser. 1994. Cloning of a human CXC chemokine receptor isolated from human spleen. J. Biol. Chem. 269:232–237.

26. Bleul, C.C., L. Wu, J.A. Hoxie, T.A. Springer, and C.R. Mackay. 1997. The HIV coreceptors CXCR4 and CCR5 are differentially expressed and regulated on human T lymphocytes. Proc. Natl. Acad. Sci. USA. 94:1925–1930.

27. Heath, H., S. Qiu, P. Rao, L. Wu, G. LaRosa, N. Kassem, P.D. Ponath, and C.R. Mackay. 1997. Chemokine receptor usage by human eosinophils. The importance of CCR3 demonstrated using an antagonistic monoclonal antibody. J. Clin. Invest. 99:178–184.

28. Endres, M.J., P.R. Clapham, M. Marsh, M. Ahuja, J. Davis-
Pascual, V., Y.-J. Liu, A. Magalski, O. de Bouteiller, J. Banchereau, and J.D. Capra. 1994. Analysis of somatic mutation in five B cell subsets of human tonsil. J. Exp. Med. 183:329–339.

Liu, Y. J., D.E. Joshua, G.T. Williams, C.A. Smith, J. Gordon, and I.C.M. MacLennan. 1989. Mechanism of antigen-driven selection in germinal centres. Nature 342:929–931.

Howard, T.M., and D. Wang. 1987. Calcium ionophore, phorbol ester, and chemotactic peptide-induced cytoskeleton reorganization in human neutrophils. J. Cell Biol. 104:2165–2171.

Howard, T.H., and W.H. Moyer. 1984. Chemotactic peptide modulation of actin assembly and locomotion in neutrophils. J. Cell Biol. 89:1265–1271.

Arpin, C., J. Dechanet, C. Van Kooten, P. Merville, G. Grouard, F. Briere, J. Banchereau, and Y.-J. Liu. 1995. Generation of memory B cells and plasma cells in vitro. Science 268:720–722.

Schultze, J.L., A.A. Cardoso, G.J. Freeman, M.J. Seamon, J. Howard, and W.H. Meyer. 1984. Chemotactic peptide modulation of actin assembly and locomotion in neutrophils. J. Cell Biol. 89:1265–1271.

Arpin, C., J. Dechanet, C. Van Kooten, P. Merville, G. Grouard, F. Briere, J. Banchereau, and Y.-J. Liu. 1995. Generation of memory B cells and plasma cells in vitro. Science 268:720–722.