CXCR5-Transduced Bone Marrow-Derived Dendritic Cells Traffic to B Cell Zones of Lymph Nodes and Modify Antigen-Specific Immune Responses

Meng-Tse Wu and Sam T. Hwang

*J Immunol* 2002; 168:5096-5102; doi: 10.4049/jimmunol.168.10.5096

http://www.jimmunol.org/content/168/10/5096
CXCR5-Transduced Bone Marrow-Derived Dendritic Cells Traffic to B Cell Zones of Lymph Nodes and Modify Antigen-Specific Immune Responses

Meng-Tse Wu¹ and Sam T. Hwang²

Skin-derived migratory dendritic cells (DC), in contrast to bone marrow-derived DC (BMDC), express CXCR5, respond to the chemokine CXC ligand 13 (CXCL13) in vitro, and are capable of migrating to B cell zones (BCZ) in lymph nodes (LN) in vivo. Herein, we analyzed the surface phenotype of skin-derived migratory DC and found that 15–35% of MHC class II⁺ cells showed high levels of expression of CXCR5 but expressed low levels of DEC205, a suggested characteristic of dermal-type DC in mice. To study the effects of CXCR5 on the trafficking dynamics of DC, we stably expressed CXCR5 in BMDC by retroviral gene transduction. CXCR5 was detected by flow cytometry on transduced cells, which responded to CXCL13 in vitro in chemotaxis assays (3-fold over nontransduced BMDC, \( p < 0.01 \)). When injected into the footpads of mice, ~40% of injected CXCR5-BMDC were observed in BCZ of draining LN. Mice were vaccinated with CXCR5- and vector-BMDC that were pulsed with keyhole limpet hemocyanin (KLH) to induce Ag-specific cellular and humoral immune responses. Mice injected with CXCR5-BMDC (vs vector-BMDC) demonstrated marginally less footpad swelling in response to intradermal injection of KLH. Interestingly, significantly higher levels of KLH-specific IgG (\( p < 0.05 \)) and IgM (\( p < 0.01 \)) were found in the serum of mice injected with CXCR5-BMDC compared with mice injected with vector-transduced BMDC. Thus, CXCR5 is predominantly expressed by dermal-type DC. Moreover, CXCR5 directs BMDC to BCZ of LN in vivo and modifies Ag-specific immune responses induced by BMDC vaccination. The Journal of Immunology, 2002, 168: 5096–5102.

To efficiently stimulate a humoral response, Ag-presenting dendritic cells (DC)¹ must leave peripheral tissue via afferent lymphatic vessels and eventually enter distinct anatomical areas of draining lymph node (LN). In the classical DC-T cell-B cell triad relationship, DC activate and expand T cells, which in turn induce B cell growth and Ig secretion (1). There is, however, strong evidence indicating that DC may directly interact with and regulate the function of B cells (2). The data also suggest that different DC subsets have specific regulatory roles in B cell differentiation and development. For instance, in vitro methods for culturing human DC from CD34⁺ hemopoietic progenitors yield both CD1a⁺ DC that possess Birbeck granules and resemble epidermal DC or Langerhans cells (LC) and CD14⁺, Birbeck granule-negative DC that resemble dermal or interstitial DC (3). Interestingly, although both LC- and dermal-type DC can induce the differentiation of memory B cells, only dermal-type DC are able to induce naive B cells to differentiate into IgM-secreting cells (4). These results suggest that dermal-type DC are more likely to be critical in launching primary B cell responses (2).

Other types of DC (i.e., ex vivo-generated follicular DC (FDC) and germinal center DC (GCDC)) also have direct effects on B cell growth and development (2, 5–7). Furthermore, DC capture and transfer unprocessed Ags to naive B cells to initiate a specific humoral response in vivo (8). Thus, in addition to the critical role of DC in the initiation of cellular immune responses (9), these studies support the direct involvement of DC in the initiation and regulation of humoral immune responses.

For DC to regulate B cell function, we hypothesize that physical contact between DC and B cells is likely to be important and that DC must traffic to B cell zones (BCZ) of secondary lymphoid organs. There are already several lines of evidence that support these hypotheses. DC form clusters with CD40-stimulated B cells in coculture in vitro (6), as was previously observed with DC and T cell interaction (10). Disruption of FDC-B cell clusters using mAb to LFA-1 (CD11a), VLA-4 (CD49d), ICAM-1 (CD54), or VCAM-1 (CD106) leads to apoptosis of the B cells, indicating that one or more adhesion pathways are involved in DC and B cell interactions (11). Finally, ligand-receptor interactions between CD21 on B cells and CD21 ligand (CD21L) on FDC mediate FDC accessory activity that leads to potent high affinity Ab production (12). As to whether DC can traffic to BCZ in vivo, there are several reports of DC populations that can migrate to BCZ in vivo (13, 14).

It has already been established that chemokines and chemokine receptors play specific roles in DC migration (15, 16). For example, DC expression of CCR7 is necessary for lymphatic entry and migration to the LN (17–19), and CCR6 likely influences the migration of DC in Peyer’s patches (20, 21). CXC ligand 13 (CXCL13, also known as B lymphocyte chemoattractant or BLC) is highly expressed in BCZ of secondary lymphoid organs (22–24).
Recent results showed that CXCL13 and CXCR5 are required for B cell homing to the follicles of the LN and for the development of most LN and Peyer’s patches (25, 26). We previously showed that ex vivo–generated, skin-derived migratory DC also expressed CXCR5, responded to CXCL13 in vitro, and migrated to BCZ as well as T cell zones (TCZ) in draining LN in vivo (14). By contrast, bone marrow–derived DC (BMDC) lacked CXCR5 expression, did not respond to BLC in vitro, and migrated exclusively to TCZ in draining LN (14, 27). Herein, we transduced BMDC with CXCR5 to determine whether CXCR5 was involved in promoting migratory skin-derived DC to BCZ. We found that CXCR5–BMDC responded to CXCL13 in vitro, colocalized to BCZ in vivo, and, importantly, altered the immune response of the mouse as a consequence of their new trafficking pattern.

Materials and Methods

Animals and generation of BMDC

Female BALB/c mice (8–12 wk old) raised under specific-pathogen-free conditions were used in all experiments, which were approved by the National Cancer Institute Animal Care and Use Committee. BMDC were cultured as described previously with modification (28). Briefly, bone marrow was flushed from the long bones of the limbs, filtered through a nylon mesh, and depleted of RBC with ammonium chloride. Bone marrow cells (BMC) were incubated for 30 min with a panel of mAb to murine CD45R/CD45R0 (RA3-6B2, rat IgG 2a), CD90.2 (53-2.1, rat IgG2a), and I-A/I-E (29, rat IgG2b, Becton Dickinson, San Jose, CA) for the depletion of lymphocytes, granulocytes, and MHC class II-positive cells. Ab-labeled cells were then incubated with sheep anti-rat IgG-coated magnetic beads (Dynal, Oslo, Norway). Lineage marker-negative cells were plated in six-well culture plates (4 × 10^6 cells/ml; 1 ml/well) in complete RPMI medium (cRPMI) with following components: RPMI 1640 (Life Technologies, Gaithersburg, MD); 5% heat-inactivated FCS; 50 μM 2-ME; 2 mM glutamine; 10 μg/ml gentamicin; 100 μg/ml streptomycin; supplemented with recombinant murine IL-4 (10 ng/ml); murine GM-CSF (10 ng/ml) (PeproTech, Rocky Hill, NJ); and murine Flt3-L (R&D Systems, Minneapolis, MN).

Construction of retroviral vectors and retroviral gene transduction

The vector MSCV-IRES-GFP (MIG, a gift from M. Mackey, National Institute of Arthritis and Infectious Diseases, National Institutes of Health, Bethesda, MD), made by insertion of the internal ribosomal entry site (IRES)-green fluorescent protein (GFP) sequence into MSCV 2.2 (29), was cotransfected with 20 μg of either MIG or CXCR5-MIG and 20 μg of pVSV-G envelope protein vector (Clontech) using FuGene 6 transfection reagent (Roche Diagnostics, Indianapolis, IN) to produce MIG and CXCR5-MIG retrovirus in supernatant. For retroviral-mediated transduction, 3 ml of retrovirus supernatant (freshly harvested or thawed from frozen stock) were filtered through a 0.45-μm pore low protein binding filter (Millipore, Bedford, MA), supplemented with Polybrene (8 μg/ml; Sigma, St. Louis, MO) and added together with BMC in six-well plates on days 0, 1, and 2. Transduction was enhanced by centrifugation at 2500 × g at 25°C for 1 h. Supernatant was removed after centrifugation, and BMC were resuspended with complete medium supplemented with cytokines. Medium was replenished with cytokines on day 5, and nonadherent cells were harvested by gentle pipetting on day 7. BMDC surface phenotype was examined by flow cytometry with PE-conjugated murine CD11c mAb (HL3, hamster IgG; BD Pharmingen) and anti-murine CXCR5 (a gift from J. G. Cyster, University of California, San Francisco, CA). CXCR5 transduction efficiency was also examined by expression of GFP by fluorescence microscopy and flow cytometry on day 7.

Real-time quantitative RT-PCR

RNA extraction and reverse transcription were performed as described (14). Real-time quantitative PCR (Perkin-Elmer ABI7700) was performed with duplicate samples using SybrGreen dye and the murine primer pairs for detection of CXCR5 dsDNA, 5′-AAGCTCTTTACCACAGTGGCAC-3′ and 5′-GGAAACGGGGTGAGAACCAC′-3′. Murine G3PDH primer pairs (5′-CGTGTTCTACCCCCAGTGT-3′ and 5′-TGTCATACTTGCAGGATTCCTC-3′) were used as an internal control for loading. All primer pairs were designed to give products between 65 and 75 bp long. All relative quantifications were performed using the relative expression method of Pfaffl (30). The 2^-ΔΔCt^ method was used to calculate fold changes in expression.

In vitro chemotaxis assay

Transduced (MIG or CXCR5-MIG) or nontransduced BMDC were used in chemotaxis assays as previously described (14). Cells (20,000 cells in 25 μl cRPMI) were placed on top of the 5-μm pore size filters in duplicate, whereas cRPMI with and without chemokines were placed into the lower chamber. After 3 h at 37°C, migrated cells that had fallen to the bottom of the plate were photographed using a 4× objective. Three random views from each of two wells were counted using Image Pro Plus (Media Cybernetics, Silver Spring, MD). Three independent experiments were performed with similar results.

In vivo CXCR5-BMDC homing assay

CXCR5-BMDC or vector-BMDC (300,000 cells in 50 μl Hanks’ buffer with 0.1% BSA) were either labeled with PKH67 (Sigma) membrane dye according to manufacturer’s instructions or left unlabeled and injected into the left hind footpad of mice. The left popliteal LN was removed 24, 48, or 72 h later; sectioned (6 μm); air-dried without fixation; and stained with biotin-conjugated CD45R/B220 mAb (BD Pharmingen) followed by streptavidin-Cy3 to identify BCZ. All analysis was performed with a Nikon PCM2000 laser scanning confocal microscope and Adobe Photoshop 6.0 software (Adobe Systems, Mountain View, CA).

Induction of Ag-specific immune responses

Induction of Ag-specific cellular and humoral immune responses using transduced BMDC was performed as previously described (32) with the following modifications. For cellular immune responses, CXCR5-BMDC or vector-BMDC were harvested on day 7, washed with PBS, and resuspended in cRPMI in the presence or absence of keyhole limpet hemocyanin (KLH; 100 μg/ml; Sigma) for 8 h. Mice (four groups, five mice in each group) were sensitized by s.c. injection (3 × 10^6 cells/animal) of CXCR5-BMDC or vector-BMDC, either pulsed with KLH or not, into the right flank. In another group serving as a positive control, animals received a s.c. injection of KLH in CFA (2 mg/ml, 100 μl/animal) instead of BMDC. Six days later, the mice were challenged with s.c. injection of KLH (50 μg/animal) in 30 μl PBS into the left footpad and 30 μl PBS alone into the right footpad. Footpad thickness of both feet was measured before and 24 h after KLH challenge by a blinded, experienced observer using a thickness gauge (Mitutoyo, Kawasaki, Japan). Footpad swelling response was calculated as the swelling (thickness after challenge) minus the thickness of the left footpad that received Ag minus the baseline swelling of the right footpad that received PBS.

For humoral immune responses, mice (five mice/group) of the indicated strains were immunized (300,000 cells per animal) of KLH-pulsed or nonpulsed CXCR5-BMDC or vector-BMDC. One week after the third and fifth injections, serum samples were collected, and relative concentrations of KLH-specific IgM, IgG1, IgG2a, and IgG2b were measured using a Mouse Hybridoma Subtyping ELISA Kit (Roche). OD_{405} ELISA readings from serum samples at 1/500 dilution (based on preliminary experiments showing OD_{405} readings between 0.1 and 0.6 from tested and reference serum samples at this dilution) were compared with reference serum samples (1/500 dilution) that were collected and pooled from five mice 10 days after single immunization with a s.c. injection of KLH in CFA (2 mg/ml, 100 μl/animal).

Statistical analysis

Means and p values (Student’s t test, parametric, two-tailed analysis) for chemotaxis assays and immune response induction were obtained using Excel (Microsoft, Redmond, WA).

Results

MHC class II^high/dendritic and epithelial cell-205 (DEC205)^low DC derived from skin explants express CXCR5

Our previous results using quantitative RT-PCR methods demonstrated that there was detectable expression of CXCR5 mRNA from cells migrating out of murine skin explants. Most of these
cells (>85%) were DC as demonstrated by characteristic morphology and uniformly high expression of MHC class II and costimulatory molecules. Recently, Henri et al. (33) have shown that migratory skin-derived DC, such as the ones we previously isolated, likely consist of at least two subpopulations. One subpopulation has high expression of both MHC class II and a lectin-like protein called DEC205 (34), both of which are consistent with features of epidermal DC (i.e., LC). The other subpopulation showed equally high expression of MHC class II but displayed lower levels of DEC205, and were thus identified as dermal DC. Using the expression of DEC205 as a basis for distinguishing LC from dermal DC, we stained migratory skin-derived DC with anti-CXCR5 Ab and I-Ad (or DEC205) (Fig. 1) and analyzed expression by two-color flow cytometry. From 15 to 35% of I-Ad(high) cells demonstrated expression of CXCR5 in two different experiments (Fig. 1A). By comparison, BMDC, which express very low levels of CXCR5 by RT-PCR (14), showed <6% CXCR5-positive cells (Fig. 1A). Whereas the majority of I-Ad(high) cells were DEC205 positive, CXCR5(high) cells were low in DEC205 expression (Fig. 1B), suggesting that they were likely to be dermal-type DC rather than LC.

Transduction of BMDC with CXCR5

From our earlier work, BMDC did not express CXCR5 and did not migrate to BCZ (14). To determine whether the absence of CXCR5 in the BMDC could account for their selective migration to TCZ, we transduced BMDC with the gene for murine CXCR5 using the MIG-retroviral vector. CXCR5-MIG or MIG-transduced BMC started to express GFP as early as 24 h after transduction, which could be conveniently observed by fluorescence microscopy (Fig. 2A). When examined by flow cytometry 7 days after first transduction, both CXCR5-BMDC and vector-BMDC demonstrated CD11c expression (~83%) (Fig. 2B). Transduction efficiencies of CXCR5 from several experiments were consistently ~40% by GFP expression (Fig. 2C) and ~60% by CXCR5 expression (Fig. 2D) on day 7. The discrepancy in expression between upstream (cap-dependent) and downstream (IRES-dependent) coding regions had also been previously described from 0 to 75% due to different translational mechanisms (35).

**FIGURE 1.** CXCR5 expression by skin-derived migratory DC (migDC). BMDC were isolated as described in Materials and Methods, whereas skin-derived migratory DC were isolated after 3 days of culture in a skin explant system (14). For flow cytometric analyses, an anti-mouse CD16/32 mixture (BD PharMingen) was added to cells to prevent nonspecific Fc-mediated binding. A, Cells were then stained with FITC-anti-MHC class II (BD PharMingen; clone 2G9, rat IgG2a) and rabbit anti-mouse CXCR5 or rabbit IgG control followed by a PE-labeled, goat anti-rabbit IgG secondary Ab (Caltag Laboratories, Burlingame, CA). For migratory DC and BMDC, cells were first gated by forward scatter (FSC) and side scatter and then by those cells with high expression of I-Ad (gates R2 and R4). Those cells within R2 (migDC) and R4 (BMDC) were then analyzed for CXCR5-PE expression in the histograms as shown. Gray filled line, Rabbit IgG control. Dark solid unfilled line, Anti-CXCR5. The percent of CXCR5-positive (Pos.) cells in relationship to IgG control is indicated. B, Skin-derived migratory DC were stained for anti-CXCR5 (as above) and biotinylated DEC205 (Cedarlane Laboratories, Hornby, Ontario, Canada) followed by streptavidin-FITC.

**FIGURE 2.** Retroviral transduction of BMDC with CXCR5. The CXCR5 gene was transduced into BMDC as described in Materials and Methods. A, CXCR5-BMDC expressed GFP on Day 4 after transduction. B, By flow cytometry, >80% of day 7 CXCR5-BMDC and vector-BMDC expressed the CD11c phenotypic marker. Transduction efficiency of CXCR5-BMDC on day 7 was ~40% by GFP expression with nontransduced BMDC as a control (C) and 60% by CXCR5 expression (D). E, CXCR5 mRNA expression (by real-time RT-PCR) from transduced BMDC is quantified relative to CXCR5 expressed by nontransduced BMDC. M1, Marker used to define positive staining.
BMDC was found to express 41-fold more CXCR5 than nontransduced BMDC using real-time quantitative PCR. CXCR5 mRNA in nontransduced BMDC, vector-BMDC, and CXCR5-BMDC migrate in response to CXCL13 in vitro. Thus, BMDC can be efficiently transduced with CXCR5 by retroviral gene transduction with an overall efficiency of ~50%.

**CXCR5-BMDC migrate in response to CXCL13**

To determine whether CXCR5 was functional in the transduced BMDC, we performed chemotaxis assays using murine CXCL13 or CCL21 (also known as secondary lymphoid tissue chemokine (36)) as chemoattractants. As shown in Fig. 3, CXCR5-BMDC responded to CXCL13 with a ~3-fold increase in migration over either vector-BMDC or nontransduced BMDC. CXCR5-BMDC and vector-BMDC, however, showed comparable levels of response to CC ligand 21 (CCL21) along with nontransduced BMDC, indicating normal expression and function of CCR7 in retroviral-transduced BMDC. The response of CXCR5-BMDC to CXCL13 was similar to that observed in skin-derived migratory DC (14). Thus, CXCR5-BMDC, but not BMDC or vector-BMDC, efficiently migrated to CXCL13 in vitro. Furthermore, the response of the transduced BMDC to CCL21 (acting via CCR7) suggested that the BMDC had an activated phenotype (19).

**In vivo homing of CXCR5-BMDC**

Given that nontransduced, wild-type BMDC migrated exclusively to TCZ in draining LN (14, 27), we sought to determine whether CXCR5 gene transduction would alter the migration dynamics and homing pattern of BMDC within draining LN. First, we labeled day 7 CXCR5- or vector-BMDC with a stable membrane dye, PKH67 (green), and injected them into the footpad of mice. After 24, 48, or 72 h, draining popliteal LN were removed, sectioned, and stained with an anti-B cell mAb and rephotographed to delineate BCZ (Fig. 4, A and D). Labeled vector-CXCR5 DC migrated exclusively to T cell zones (Fig. 4, B and C), whereas a significant proportion of CXCR5-BMDC (green) migrated to BCZ in addition to TCZ (Fig. 4C). At higher magnification, CXCR5-BMDC in the BCZ showed dendritic morphology (Fig. 4C, inset). CXCR5-BMDC were also present at the border zones between BCZ and TCZ (not shown). The percentage of CXCR5-BMDC detected within the BCZ was 39 ± 3.4% (SD) at 72 h. CXCR5-BMDC migration to BCZ occurred in all sections sampled from early (24 h) to late (72 h) time points. Because the CXCR5-BMDC injected in the experiment described above did not uniformly express CXCR5 and GFP (as shown in Fig. 2), we repeated the experiment with CXCR5-BMDC that were selected by flow cytometric sorting for a positive GFP signal (>90% GFP+) and found 46 ± 7% of CXCR5-BMDC in BCZ at 48 h after injection, a proportion that was not statistically different (p = 0.1) compared with previous experiments involving nonsorted BMDC. Unstained CXCR5-BMDC expressing GFP were also observed in BCZ using alternative staining protocols (data not shown). Thus, CXCR5-BMDC, but not vector-BMDC, were able to effectively migrate to BCZ in draining LN in vivo.

**Ag-specific immune responses induced by CXCR5-BMDC**

To address whether trafficking of CXCR5-BMDC to BCZ could alter Ag-specific immune responses, we pulsed the CXCR5-BMDC or vector-BMDC with KLH and injected them into mice as described in Materials and Methods to induce KLH-specific cellular or humoral immune responses. Cellular immune responses were demonstrated by footpad swelling after challenging the animals by injecting KLH intradermally into the footpads. As shown in Fig. 5, animals immunized with CXCR5-BMDC had 23% less footpad swelling (p < 0.05) than those injected with vector-BMDC. When GFP-sorted (as described above) CXCR5-BMDC were used to immunize mice for cellular immune responses, a similar difference of 27% less swelling in the CXCR5-BMDC-injected mice was observed. Humoral immune responses were measured by a quantitative ELISA-based assay for KLH-specific Ig after 3 and 5 wk of weekly injections with KLH-pulsed DC. In response to weekly injections of KLH-pulsed BMDC, animals immunized with CXCR5-BMDC produced more IgM (2.9-fold OD reading level, p < 0.05) and IgG2a (2-fold, p < 0.01) compared with vector-BMDC after the third injection and more IgM (4-fold, p < 0.01), IgG (1.44-fold, p < 0.05), IgG2a (2.1-fold, p < 0.01), and IgG2b (2.6-fold, p < 0.05) after the fifth injection (Fig. 6A).
GFP-sorted CXCR5-BMDC were also used for assessment of humoral immune responses in a similar protocol except that Ig was assessed 6 days after two weekly injections of CXCR5-BMDC. Titration of sera from immunized mice showed significantly more KLH-specific IgG (Fig. 6C) and IgM (Fig. 6B) from mice immunized with CXCR5-BMDC compared with vector-transduced BMDC at the lower serum dilution ranges, similar to results obtained with unsorted BMDC in Fig. 6A. Thus, although there were only modest changes in the cellular immune response in mice immunized with CXCR5-BMDC, a consistent elevation of IgM and IgG were observed in CXCR5-BMDC-vaccinated mice compared with those vaccinated with vector-transduced BMDC.

**Discussion**

In our previous study describing the expression of CXCR5 by migratory skin-derived DC, we were able to show that these DC could localize in BCZ. We could not, however, demonstrate the in vivo immunologic outcome of this phenomenon nor could we determine whether dermal DC, LC, or both populations expressed CXCR5. In the present study, we have shown that expression of CXCR5 is associated with skin-derived DC that display features of dermal DC. Furthermore, we have transduced BMDC, which normally do not express functional levels of CXCR5, with sufficient CXCR5 to alter their migration patterns both in vitro and in vivo. Importantly, after immunization of mice in vivo with CXCR5-BMDC, humoral immune responses were substantially increased relative to vector-BMDC. Our results demonstrated that expression of CXCR5 is sufficient to change the trafficking dynamics of BMDC and to modify Ag-specific host immune responses induced by BMDC vaccination.

The studies of Caux and colleagues (2) have shown that certain subsets of DC can directly stimulate B cells. In particular, interstitial/dermal-type, but not LC-type, DC have potent B cell-stimulatory properties (4). This may partially explain the high efficiency of Th2-type responses generated by the intradermal route of plasmid DNA administration (37). Although clear markers for dermal vs epidermal DC are available for human skin-derived DC and
for DC derived from human CD34+ hematopoietic progenitor cells (3, 4), these two types of skin-derived DC have previously been virtually indistinguishable in mice (38). A recent report by Henry et al. (33), however, demonstrated that dermal DC and LC derived from murine skin explants were both similarly high in MHC class II but that dermal DC showed low expression of DEC205, a lectin domain-containing protein that may participate in the endocytosis of potential Ags. Our observation showing that CXCR5 is primarily expressed by DEC205low migratory skin DC suggests that it is the dermal-type DC subpopulation that primarily expresses CXCR5 (Fig. 1). The proportion of I-Ad<sup>αβ</sup> cells that express CXCR5 appears to vary depending on the skin preparations, perhaps because the preparative procedure requires scraping and removing variable amounts of dermis from the mouse ear. Our current results suggest that the dermal component of the migratory skin DC expressed CXCR5 and migrated to BCZ in our previous study (14). Thus, dermal DC may use CXCR5 as a migratory mechanism to migrate to BCZ, where they may play a critical role in stimulating naïve B cells (2).

To date, several subsets of DC have been found resting within or migrating to the lymphoid follicles. These include FDC (39), CD3<sup>+</sup> CD4<sup>+</sup> CD11c<sup>+</sup> GCDC (2, 40), a population of CD11c<sup>+</sup> DC characterized by binding to a mannose receptor fusion protein (13), as well as a subpopulation of migratory skin-derived DC (14). Although the trafficking mechanisms for FDC and GCDC homing to lymphoid follicles are still unclear, our current studies suggest that CXCR5 may be critical for the migration of DC subsets to BCZ. It has already been established that CXCR5/CXCL13 represent an important pathway for the migration of B cells to lymphoid follicles (25, 26), and recent data indicate that a certain subset of T cells termed “follicular helper T cells” express CXCR5, localize to B cell follicles, and support Ab production in vitro (41). As suggested by the finding that transgenic expression of CXCL13 in pancreatic islets can lead to the formation of ectopic LN-like structures (42), it is likely that CXCR5/CXCL13 may play a role in coordinating the development of the humoral immune response by recruiting different types of CXCR5<sup>+</sup> cells, including DC subsets, to BCZ to establish a functional immune microenvironment.

We can only speculate as to why only 40–50% of CXCR5<sup>-</sup> BMDC (even those that were positively sorted for GFP expression) became localized to the BCZ. First, it is clear that CXCR5<sup>-</sup> BMDC still retain high, functional levels of CCR7 as demonstrated by their strong response to CCL21 in chemotaxis assays (Fig. 3). Because we have no effective means for down-regulating or blocking the action of CCR7 in the BMDC, a tendency to retain CXCR5<sup>-</sup>BMDC in the Tcz via CCR7 may be present. Second, the migration of DC to different areas of the LN may be one of a fine regulation based on differential levels of receptors and ligands. Although comparable with the expression of CXCR5 by dermal skin-derived DC, there is a range of CXCR5 receptor expression in our transduced cells (as well as on skin-derived migratory DC) that may affect the ultimate localization of the DC. Low and medium expression of CXCR5 may not be sufficient to allow these cells to get to the BCZ if the BMDC express high levels of CCR7.

Given the potent capacity of DC to activate T cells, DC-based vaccine strategies have been widely used to generate Th1-mediated protective immunity against cancers (43) and infectious microorganisms (44), which can be further augmented by retroviral transduction of DC with IL-12 (45). Although the decrease in cellular immune responses found in the CXCR5-BMDC vs vector-BMDC mice was statistically significant, the quantitative difference was modest. Because only one-half of CXCR5-BMDC migrated to the BCZ, the fraction that remained in the T cell zone likely interacted with naïve T cells and thus stimulated the development of cellular immune responses comparable with those of vector-BMDC.

We have shown, however, that retroviral transduction of BMDC with CXCR5 provides a feasible way to increase Ag-specific humoral immune responses. With DC-based vaccination, there has been limited success in the attempt to generate Th2-shifted or humoral immune responses using DC-based vaccination strategies. Hayashi et al. (32) had sought to induce Th2-directed immune responses using IL-4-transfected XS-106 DC, but with a 0.5–1% transfection rate, humoral responses were only modestly altered in their study. Using the approaches in the present study that resulted in >50% transduction, it is possible that higher titer, more durable humoral immune responses can be reached in future DC-based vaccination strategies by specifically altering DC migratory patterns with specific chemokine receptors.

Acknowledgments

We thank Dr. Mark C. Udey for helpful comments, Hui Fang for technical assistance, and Dr. Jason Cyster for his gift of anti-CXCR5 Ab (46).

References


