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The CC Chemokine Receptor-7 Ligands 6Ckine and Macrophage Inflammatory Protein-3β Are Potent Chemoattractants for In Vitro- and In Vivo-Derived Dendritic Cells

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Dendritic cell migration to secondary lymphoid tissues is critical for Ag presentation to T cells necessary to elicit an immune response. Despite the importance of dendritic cell trafficking in immunity, at present little is understood about the mechanisms that underlie this phenomenon. Using a novel transwell chemotaxis assay system, we demonstrate that the CC chemokine receptor-7 (CCR7) ligands 6Ckine and macrophage inflammatory protein (MIP)-3β are selective chemoattractants for MHC class II^{high} B7-2^{high} bone marrow-derived dendritic cells at a potency 1000-fold higher than their known activity on naive T cells. Furthermore, these chemokines stimulate the chemotaxis of freshly isolated lymph node dendritic cells, as well as the egress of skin dendritic cells ex vivo. Because these chemokines are expressed in lymphoid organs and 6Ckine has been localized to high endothelial venules and lymphatic endothelium, we propose that they may play an important role in the homing of dendritic cells to lymphoid tissues. ***The Journal of Immunology*****, 1999, 162: 3859–3864.**

Efficient initiation of T cell-dependent immunity is a complex sequence of events that ultimately depends upon simultaneous colocalization of Ag-presenting dendritic cells and Ag-specific CD4$^+$ or CD8$^+$ T cells in the T cell areas of lymphoid organs (1), leading to activation and expansion of rare T cell clones. Satisfaction of the requirements for this rendezvous is facilitated by the constant recirculation of naïve T cells through the lymphoid organs (2), together with the migration into lymphoid organs of Ag-presenting dendritic cells.

Dendritic cells are bone marrow-derived cells that serve a sentinel role in vivo (3, 4). Immature dendritic cells are distributed throughout many tissues; they are specialized at Ag uptake and processing, but generally express low levels of costimulatory molecules and MHC-peptide complexes on their cell surface (5, 6). Upon stimulation, e.g., by inflammatory signals or CD40 triggering, dendritic cells up-regulate costimulatory molecules, such as CD86 (B7-2), CD80 (B7-1), and CD40 (7). Furthermore, their capability to take up Ag is supplanted by a heightened ability to present Ag (5, 6). Activation of dendritic cells leads to their appearance in T cell-rich areas of secondary lymphoid organs (8), where, as mature dendritic cells, they interface with recirculating T cells. TCR-mediated recognition of specific Ag presented by these dendritic cells subsequently results in T cell priming. Thus, two parallel homing processes, T cell recirculation through lymphoid tissues and Ag-bearing dendritic cell localization to T cell areas in lymphoid tissues, are largely responsible for efficient immune surveillence.

The ability of dendritic cells to migrate from areas of Ag encounter to sites of T cell priming is fundamental to their capacity to elicit an immune response; however, the mechanisms governing this phenomenon remain largely unknown. The molecular mechanisms of T cell recirculation have been the subject of investigation for several decades, and a number of adhesion molecules participating in this process have been defined (9–11). Recently, the role of chemokines, a large family of low m.w. chemoattractant cytokines, has also been illuminated (12). 6Ckine is a recently discovered chemokine that features an unusually long carboxy-terminal tail containing two additional cysteines (13–15). It is strongly expressed in the T cell zones of lymph nodes, as well as the high endothelial venules (HEV)[2] of lymph nodes and Peyer's patches (16, 17). 6Ckine has been shown to mediate adhesion (18) as well as chemotaxis (14–17, 19) of T cells, which suggests that it may be important in lymphocyte homing. Interestingly, 6Ckine is also expressed by the endothelial cells lining lymphatic venules (or vessels) (17), suggesting a role for this chemokine in homing of dendritic cells to secondary lymphoid tissues.

Here, we show evidence that 6Ckine, as well as another CC chemokine receptor-7 (CCR7) ligand, macrophage inflammatory protein (MIP)-3β, are extremely potent inducers of in vitro- as well as in vivo-derived MHC class II^{high} B7-2^{high} dendritic cell migration. The ability of 6Ckine and MIP-3β to attract dendritic cells, combined with the expression pattern of these chemokines (13–17, 20, 21), implicates them as key factors in recruiting dendritic cells into secondary lymphoid organs.

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[2] Abbreviations used in this paper: HEV, high endothelial venules; GM-CSF, granulocyte-macrophage CSF; MIP, macrophage inflammatory protein; CCR, CC chemokine receptor; SDF, stromal cell-derived factor; m, murine.

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Materials and Methods

Cells

Bone marrow-derived dendritic cells were generated by culturing bone marrow cell suspensions (22, 23) obtained from female BALB/c mice (Taconic, Germantown, NY) in RPMI 1640 supplemented with 10% FCS, 10 mM HEPES, 1 mM sodium pyruvate, 55 μM 2-ME, L-glutamine, penicillin/streptomycin, 10 μg/ml gentamicin sulfate, 10 ng/ml granulocyte-macrophage (GM)-CSF, and 5 ng/ml IL-4 (23). Cells were cultured for 5–7 days before assay.

Freshly isolated lymph node cells were prepared by homogenizing lymph nodes from BALB/c mice in the presence of 1 mM EDTA to dissociate cell complexes. In some experiments, the cell preparation was enriched for dendritic cells by removing T and B cells using magnetic depletion.

Abs and other reagents

FITC-conjugated anti-I-A^d/I-E^d (2G9), anti-L-selectin (MEL-14), phycoerythrin-conjugated anti-CD11c (HL3), anti-CD45RB (16A), APC-conjugated mAbs directed against B220 (RA3-6B2), CD3 (145-2C11), Gr-1 (RB6-8C5), CD4 (L3T4), and biotinylated anti-B7-2 (GL-1) were from PharMingen (San Diego, CA), as were all relevant isotype control Abs. Biotinylated anti-B7-2 was detected using streptavidin-CyChrome (PharMingen). Magnetic depletions were performed using anti-CD3 (KT3; Serotec, Kidlington, U.K.), anti-B220 (RA3-6B2; PharMingen), and anti-Gr-1 (RB6-8C5; kindly provided by B. Coffman, DNAX, Palo Alto, CA), followed by incubation with anti-rat Ig Dynabeads (Dynal, Oslo, Norway). CD40 stimulation of dendritic cells was achieved using mAb 1C10 (24). All chemokines were from R&D Systems (Minneapolis, MN).

Chemotaxis

All dilutions of cells and chemokines were made in DMEM prepared with low-endotoxin water and containing 1% low-endotoxin BSA (Sigma, St. Louis, MO). Serial dilutions of chemokine were added to 24-well plates. Bone marrow-derived cells (500,000) were added to 5-μm pore size transwell inserts (Costar, Cambridge, MA). Incubation was for 90–120 min at 37°C. When lymph node cells were used, incubation time was extended to 120 min. After removal of the transwell inserts, 10^4 15-μm microsphere beads (Dynospheres; Bangs Laboratories, Fishers, IN) were added to each well, and cells and beads were transferred to tubes. "Input cells" samples were prepared by mixing 0.5×10^6 cells from the input population with 10^4 Dynospheres. Cells were stained for flow cytometry to identify $CD11c^+$ MHC class II^+ $B7\text{-}2^+$ dendritic cells, and to exclude contaminating $CD3^+$, $B220^+$, and $Gr\text{-}1^+$ (lineage markers, hereafter collectively referred to as Lin^+) cells. Naïve $CD4^+$ T cells were identified as L-$selectin^+$ $CD45RB^{high}$. Samples were analyzed using a FACScalibur (Becton Dickinson, San Jose, CA). Numbers of cells in the input and transmigrated populations were calculated as: (no. of cells acquired/no. of Dynospheres acquired) $\times$ 10^4 Dynospheres/sample. The percentage of input cells that transmigrated was calculated as: (no. of transmigrated cells/no. of input cells) $\times$ 100. Cytospins were prepared from the starting as well as the transmigrated cell populations and stained with Giemsa (Sigma).

Ex vivo emigration of dendritic cells in response to 6Ckine and MIP-3β

Ears from BALB/c mice were aseptically removed and split into dorsal and ventral halves. Both halves were cultured separately in wells of a 24-well plate in medium lacking cytokines, in the absence or presence of 10^{-8}-10^{-7} M 6Ckine or MIP-3β. After 18–48 h of culture at 37°C, emigrated cells from each ear were stained for CD11c and I-A^d/I-E^d and analyzed by flow cytometry. Cells were quantitated by adding a defined number of Dynosphere beads to each sample, and emigrated cell numbers were corrected for variations in ear weight. The ultrastructure of emigrated cells was examined in cytospin preparations.

PCR analysis

cDNA libraries prepared from resting bone marrow-derived dendritic cells or bone marrow-derived dendritic cells stimulated overnight with anti-CD40 mAb were subjected to PCR amplification. Plasmid DNA encoding murine (m) CCR6, mCCR7, or mCXCR3 served as control. Primers used were: mCCR6, 907/5′-TCAACCCCGTGTTGTATGCG-3′ (forward) and 1063C/5′-TCACTGGTCTTGCCTGGAGATGTAG-3′ (reverse); mCCR7, 679/5′-CAGATGGTTTTTGGGTTCCTAGTG-3′ (forward) and 914C/5′-TTGAGCTGCTTGCTGGTTTCGCAG-3′ (reverse); and mCXCR3, 864/5′CTGTGGTCGAAAAAGCCACG-3′ (forward) and 1066C/5′-AGGATGATTCTCTCCGTGAAGATG-3′ (reverse). β-actin was amplified as an internal control. PCR conditions were: 94°C for 2 min, followed by 25 cycles of 94°C for 1 min, 55°C for 2 min, 72°C for 3 min, and 1 cycle of 72°C for 10 min. PCR products were resolved on a 1.2% agarose gel.

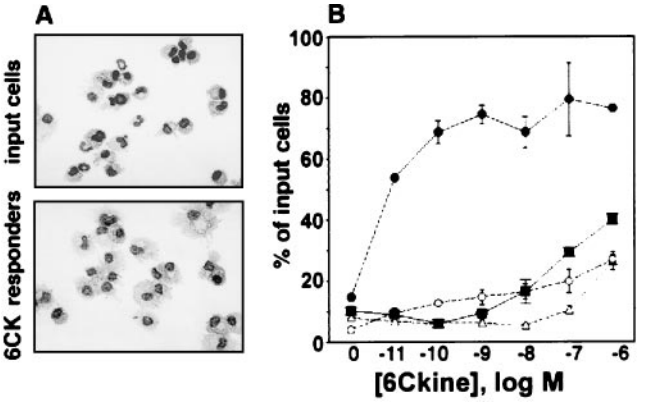

FIGURE 1. Characterization of the bone marrow-derived cells that respond to 6Ckine. *A*, Cytospin preparations from bone marrow-derived cells cultured in GM-CSF and IL-4 for 5–7 days and then tested in transwell chemotaxis assays for their response to 6Ckine. *Top panel*, Starting population applied to the upper well. *Lower panel*, Morphology of cells that have migrated toward a 6Ckine (10^{-10} M) gradient. Dendritic cells are greatly enriched in this population. Original magnification was ×400. *B*, The chemoattractant effect of 6Ckine on MHC $classII^{high}$ $B7\text{-}2^{high}$ dendritic cells surpasses its effects on MHC $classII^{low}$ $B7\text{-}2^{low}$ dendritic cells, as well as Lin^+ cells and naïve $CD4^+$ cells. Bone marrow-derived cells or freshly isolated lymph node cells were tested in parallel in transwell chemotaxis assays in which an increasing concentration of 6Ckine was added to the lower well. The transmigrated populations were stained with mAbs to identify $CD11c^+$ MHC class II^{high} $B7\text{-}2^{high}$ dendritic cells (closed circles), MHC $classII^{low}$ $B7\text{-}2^{low}$ dendritic cells (open circles), $CD3^+$/$B220^+$/$Gr\text{-}1^+$ cells (open triangles), or, in the case of lymph node cells, L-$selectin^+$ $CD4^+$ T cells (closed squares) and analyzed by flow cytometry. Data are presented as the mean ± SD of triplicate wells, and the results are plotted as the percent of each cell subset present in the starting population that transmigrated.

Results

6Ckine selectively attracts dendritic cells

As an initial approach to better understand the chemokines potentially involved in dendritic cell migration in vivo, we assessed the chemotactic response of bone marrow-derived dendritic cells in vitro. Bone marrow-derived cells cultured for 5–7 days with GM-CSF and IL-4 were assayed for their ability to migrate across transwells in response to chemokines. Subpopulations of starting and transmigrated cells were then identified and enumerated by flow cytometry. This assay system offers several benefits over other approaches such as Boyden chamber assays, in that 1) large numbers of cells can be efficiently and objectively quantitated, and 2) subsets of cells can be identified without extensive purification procedures.

Because of these advantages, bone marrow cells were not subjected to any depletion regimen in advance of assaying chemotactic responses, and the population assayed contained a mixture of several cell types. Dendritic cells were identified by their distinct morphology in cytospin preparations (Fig. 1*A*, *top*) and by their MHC class II^+ $CD11c^+$ phenotype (typically ~25–40% of the total cell population as assessed by flow cytometry; data not shown); polymorphonuclear cells expressing B220 and/or Gr-1 (Lin^+) were also present (Fig. 1*A*, *top*, and data not shown).

The recently identified CC chemokine 6Ckine (13–15) was able to efficiently and selectively chemoattract a significant proportion of bone marrow-derived cells. The population that responded to 1

ng/ml 6Ckine was enriched in dendritic cells (Fig. 1*A*, *bottom*) and was 70–75% CD11c$^+$Lin$^-$ (data not shown). The dendritic cells generated from bone marrow progenitors were heterogeneous for their expression of MHC class II and B7-2, which are up-regulated on activated or mature dendritic cells (3). Whereas the input DCs were 60–70% MHC class II^{high} B7-2^{high} and 30–40% MHC class II^{low} B7-2^{low}, the DCs responding to 6Ckine were dramatically enriched for MHC class II^{high} B7-2^{high} cells, which were typically 90% (data not shown). 6Ckine was a potent chemoattractant for MHC class II^{high} B7-2^{high} dendritic cells over a broad concentration range (Fig. 1*B*), routinely attracting 60–90% of this subset of dendritic cells at optimal concentrations (Fig. 1*B*). In contrast, only a small percentage of MHC class II^{low} B7-2^{low} dendritic cells was found in the responding population, and the effective concentration range was more limited. Incubation of dendritic cells with 6Ckine did not up-regulate class II MHC or B7-2 expression (data not shown), ruling out the possibility that MHC class II^{low} B7-2^{low} dendritic cells were attracted and subsequently became MHC class II^{high} B7-2^{high}. The specificity of 6Ckine for MHC class II^{high} B7-2^{high} dendritic cells was further illustrated by the observation that contaminating Lin$^+$ cells were unresponsive to 6Ckine, except at high concentrations (Fig. 1*B*).

Previous reports describing the chemoattractant abilities of 6Ckine have highlighted its effectiveness in T cell migration (13, 16, 17). In agreement with earlier studies, naïve CD4$^+$ T cells responded to 6Ckine in the range of 10^{-6}–10^{-7} M (Fig. 1*B*). It is striking that this concentration was 1,000–10,000 times greater than that needed to elicit a response from bone marrow-derived dendritic cells.

CD40 ligation leads to up-regulation of costimulatory molecules and activation of dendritic cells (25). To determine whether CD40-mediated activation altered dendritic cell responsiveness to 6Ckine, 5-day bone marrow-derived cultures were depleted of T cells (CD3$^+$), B cells (B220$^+$), and most Gr-1$^+$ cells (primarily granulocytes) and incubated overnight in the presence or absence of anti-CD40 mAb. While CD40 stimulation increased the proportion of dendritic cells that were MHC class II^{high} B7-2^{high}, the percentage of MHC class II^{high} B7-2^{high} dendritic cells responding to 6Ckine was unchanged (data not shown). Therefore, it seems likely that the MHC class II^{high} B7-2^{high} dendritic cells present in unmanipulated cultures represented "mature" dendritic cells that are phenotypically and functionally (with respect to chemotaxis) equivalent to CD40-stimulated dendritic cells.

The CCR7 ligands 6Ckine and MIP-3β are potent dendritic cell chemoattractants

Several other chemokines have been shown to chemoattract dendritic cells, such as stromal cell-derived factor (SDF)-1α (26, 27) and MIP-1α (26, 28, 29), and were therefore compared with 6Ckine in their ability to trigger dendritic cell chemotaxis. In agreement with previously published results, MHC class II^{high} B7-2^{high} dendritic cells responding to human SDF-1α exhibited a more typical bell shaped curve, in contrast to the extended plateau observed in response to 6Ckine (Fig. 2). Chemotaxis toward murine MIP-1α was weak or absent. Both SDF-1α and MIP-1α were capable of attracting a small percentage of MHC class II^{low} B7-2^{low} dendritic cells. The dose-dependence of the response to SDF-1α mirrored that of the MHC class II^{high} B7-2^{high} dendritic cells, while MIP-1α was effective at 10 ng/ml (data not shown).

6Ckine shares the chemokine receptor CCR7 with MIP-3β (21, 30). Accordingly, we investigated the ability of MIP-3β to attract bone marrow-derived dendritic cells. Like 6Ckine, and in contrast to SDF-1α and MIP-1α, MIP-3β attracted dendritic cells over a broad range of concentrations (Fig. 2) and was similar to 6Ckine with respect to its specificity for MHC class II^{high} B7-2^{high} dendritic cells (data not shown).

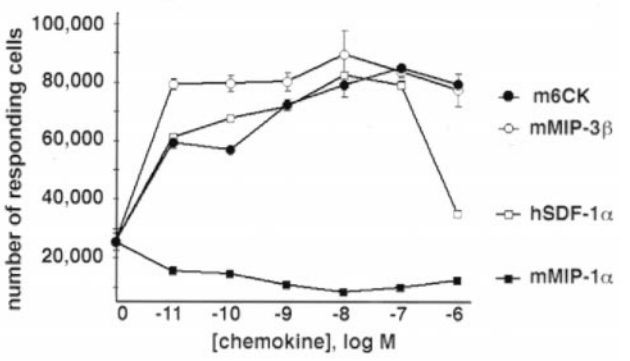

FIGURE 2. Comparison of 6Ckine (m6CK) and MIP-3β chemotactic activity on bone marrow-derived dendritic cells with that of other previously described dendritic cell chemoattractants, SDF-1α and MIP-1α. Dendritic cells from 7-day cultures were tested in transwell chemotaxis assays in which increasing concentrations of individual chemokines were added to the lower well. The transmigrated populations were stained with mAbs directed against CD11c, MHC class II, and B7-2 to identify dendritic cells and analyzed by flow cytometry. Only the data for MHC classII^{high} B7-2^{high} dendritic cells are shown. Data are presented as the mean ± SD of triplicate wells.

Checkerboard analysis

To investigate whether the activity of 6Ckine, MIP-3β, and SDF-1α was chemotactic or chemokinetic, checkerboard assays were performed. When optimal concentrations of each chemokine were added to the upper, lower, or both chambers in transwell assays, we consistently observed that each chemokine elicited migration of dendritic cells in a uniform field (i.e., chemokine was present in both upper and lower chambers), but not in the presence of a negative gradient (chemokine present only in the upper chamber) (Fig. 3). However, the number of dendritic cells that migrated through transwells in a uniform field was never equivalent to that achieved in the presence of a positive gradient, suggesting that dendritic cells were sensitive to chemokine gradients. Similarly, T cells exhibited some degree of chemokinetic activity in the presence of 10^{-6} M 6Ckine (Fig. 3).

Dendritic cells express CCR7

The nearly identical response of dendritic cells to 6Ckine and MIP-3β (Fig. 2) strongly suggested that both chemokines were exerting their effects through CCR7. However, recent evidence that 6Ckine is also a ligand for CXCR3 (31) raised the possibility that either (or both) CCR7 or CXCR3 may be involved in the observed chemotaxis of dendritic cells in response to 6Ckine. To address this question, the presence of CCR7 and CXCR3 message was assessed in cDNA libraries prepared from bone marrow-derived dendritic cells cultured in the presence or absence of anti-CD40 mAb. PCR analysis revealed that both dendritic cell populations express CCR7, but not CXCR3 (Fig. 4), suggesting that chemotaxis of in vitro-derived dendritic cells in response to 6Ckine and MIP-3β is mediated through their common receptor, CCR7. However, confirmation of this awaits the availability of neutralizing anti-receptor mAbs.

6Ckine and MIP-3β attract lymph node dendritic cells

These results indicated that 6Ckine and MIP-3β are effective chemoattractants for cultured bone marrow-derived dendritic cells.

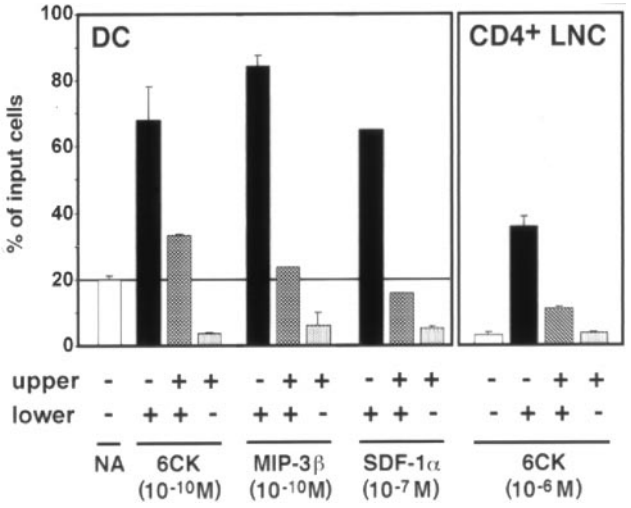

FIGURE 3. Checkerboard analysis of dendritic cell migration in response to various chemokines. Bone marrow-derived cells were tested in transwell chemotaxis assays containing various individual chemokines placed in the upper, lower, or both chambers. Optimal concentrations of each chemokine were used as determined in preliminary titration assays (see Fig. 2). Freshly isolated lymph node cells were assayed in parallel for their response to 10^{-6} M 6Ckine. The transmigrated populations were stained as described in *Materials and Methods* to identify dendritic cells (DC) and $CD4^+$ lymph node T cells ($CD4^+$ LNC), respectively, and analyzed by flow cytometry. Data are the mean ± SD of triplicate wells. The results are plotted as the percent of MHC $classII^{high}$ $B7\text{-}2^{high}$ dendritic cells or $CD4^+$ T cells present in the starting population that transmigrated.

However, to determine whether these CCR7 ligands were physiologically relevant dendritic cell chemoattractants, we assessed the chemotactic response of freshly isolated lymph node cells to 6Ckine and MIP-3β in transwell chemotaxis assays. Dendritic cells in the starting and chemoattracted populations were identified by MHC class II^+ $CD11c^+$ $B7\text{-}2^+$ staining. 6Ckine increased the transmigration of lymph node dendritic cells by 4- to 5-fold (corresponding to ~15–25% of input dendritic cells at optimal chemokine concentrations) (Fig. 5). MIP-3β was a consistent, but less potent, chemoattractant for lymph node dendritic cells (Fig. 5). The differential response to 6Ckine and MIP-3β was also observed when $CD4^+$ T cells were analyzed (data not shown). There was no response to the CXCR3 ligand, Mig (Fig. 5). Similar results were obtained with dendritic cells isolated from spleen (data not shown).

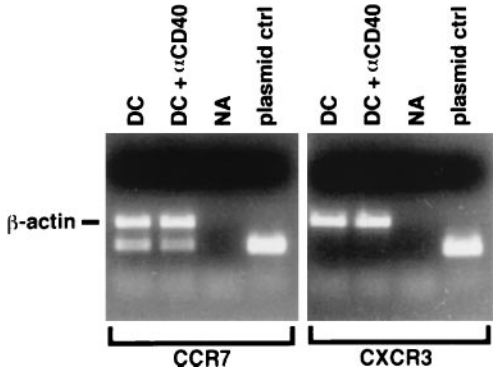

FIGURE 4. Bone marrow-derived dendritic cells express CCR7, but not CXCR3. cDNA libraries prepared from bone marrow-derived dendritic cells cultured in the absence (DC) or presence (DC + αCD40) of anti-CD40 mAb were subjected to PCR analysis using probes specific for CCR7 (*lanes 1–4*) or CXCR3 (*lanes 5–8*). Control reactions were conducted with water (NA) or using plasmids expressing the relevant chemokine receptor sequence (plasmid ctrl). β-actin served as an internal control.

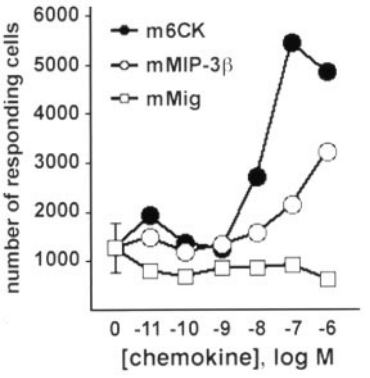

FIGURE 5. Lymph node dendritic cells respond to CCR7 ligands 6Ckine and MIP-3β. The response of freshly isolated lymph node cells toward increasing concentrations of murine (m) 6Ckine (6CK), MIP-3β, or Mig was assayed in transwell chemotaxis assays. Transmigrated cells were stained for CD11c, MHC class II, and B7-2 and analyzed by flow cytometry. Data are presented as means ± SD of triplicate wells.

6Ckine and MIP-3β attract resident skin dendritic cells ex vivo

Using a second approach to assess the ability of in vivo-generated dendritic cells to respond to 6Ckine, we took advantage of the high density of dendritic cells (Langerhans cells) in skin (7). Mouse ears were split into dorsal and ventral halves and cultured overnight in medium without cytokines, in the presence or absence of either chemokine. The emigration of MHC class II^+ $CD11c^+$ cells was consistently augmented by 6Ckine 3- to 12-fold over background levels (Fig. 6). Similar results were obtained with MIP-3β (data not shown). Consistent with a large proportion of emigrated cells staining positively for MHC class II and CD11c, cytospins indicated that most cells had a dendritic morphology (Fig. 6*B*).

Discussion

Dendritic cells are believed to be crititcal in both initiating and modulating immune responses (32). Central to their role as immune sentinels is their ability to capture, process, and transport Ag to secondary lymphoid tissues where they serve as potent APCs capable of stimulating T cells in T cell areas. Trafficking of both T cells and dendritic cells to lymphoid organs followed by precise microenvironmental localization is necessary for efficient immune

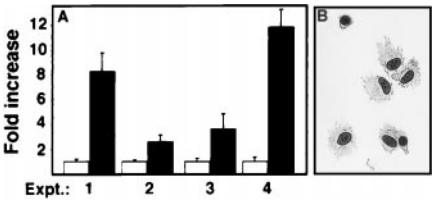

FIGURE 6. 6Ckine stimulates the egress of dendritic cells from skin. Mouse ears were split and cultured overnight in medium lacking cytokines in the absence or presence of 10^{-7}-10^{-8} M 6Ckine. *A*, Emigrated cells were harvested, stained with anti-CD11c and anti-MHC class II mAbs, and analyzed by flow cytometry. Quantitation was as described in *Materials and Methods*. Data are presented as the mean fold increase ± SD in the number of $CD11c^+$ MHC $classII^+$ cells that emigrated in the presence of 6Ckine (solid bars) compared with untreated controls (open bars) (n = 2–3 ears per condition). *B*, Cytospins were prepared from cells obtained from ears cultured overnight in 6Ckine and stained with Giemsa. Original magnification was ×400.

surveillance and is thought to be directed by chemokines (12). 6Ckine, a recently discovered CC chemokine (13–15), has been shown to be expressed by HEV in lymph nodes (16, 17), is capable of rapidly triggering integrin binding to vascular ligands (18), and is a potent chemoattractant for T lymphocytes (14–17, 19), making it a leading candidate for mediating T cell homing. 6Ckine is also expressed by endothelial cells in lymphatic venules (17), the major route of dendritic cell entry into lymph nodes (33), suggesting that it may play a role in directing dendritic cell trafficking as well.

We now report that 6Ckine is a potent chemoattractant for bone marrow dendritic cells in vitro and for MHC class II^+ $CD11c^+$ skin and lymph node dendritic cells ex vivo. We also confirm previous reports (28, 34) that MIP-3β, another CCR7 ligand, is a chemoattractant for dendritic cells generated in vitro. Furthermore, we extended these findings to show that MIP-3β is active ex vivo on MHC class II^+ $CD11c^+$ dendritic cells derived from skin and lymph nodes. The sensitivity of bone marrow-derived dendritic cells toward 6Ckine and MIP-3β is 1,000–10,000 times greater than that previously reported for T cells (13, 16, 17, 35).

Dendritic cells within the tissues efficiently capture and process Ag and, following activation, up-regulate cell surface expression of MHC class II and costimulatory molecules such as B7-2, features associated with mature dendritic cells (4). These cells then migrate to lymphoid tissues and become potent APCs capable of T cell priming. 6Ckine (and MIP-3β) preferentially chemoattracted dendritic cells expressing high levels of MHC class II and B7-2, but were relatively ineffective at attracting MHC class II^{low} B7-2^{low} dendritic cells. The selective chemoattraction of dendritic cells displaying a mature phenotype, along with the expression of 6Ckine by lymphatic endothelium, supports a potential role for 6Ckine in mediating migration of mature dendritic cells from the tissue into the draining lymph nodes.

Checkerboard analyses conducted to determine whether the response of bone marrow-derived dendritic cells toward 6Ckine and MIP-3β was chemotactic or chemokinetic showed that although the most effective migration of dendritic cells was up a gradient of either chemokine, there was a substantial amount of migration when chemokine was present in a uniform field (i.e., chemokine present in both the upper and lower wells of the transwell assay). This effect was also observed using optimal concentrations of SDF-1α. These data suggest that dendritic cells respond to chemokine-generated signals by increasing their motility (chemokinesis), but they remain sensitive to a gradient, as they were never observed to migrate down a chemokine gradient (i.e., when chemokine was only present in the upper well). In fact, the number of dendritic cells migrating down a gradient was often even less than the number spontaneously migrating in the absence of chemokine (Fig. 3).

Like many chemokines, 6Ckine binds and signals through multiple receptors; however, 6Ckine is unique in its ability to bind both a CC (CCR7; Refs. 30 and 36) and a CXC (CXCR3; Ref. 31) receptor. The nearly identical response of dendritic cells to the CCR7 ligands 6Ckine and MIP-3β strongly suggested that 6Ckine attracts dendritic cells predominantly via CCR7. PCR analysis of bone marrow-derived dendritic cells revealed expression of CCR7, but not CXCR3, supporting the hypothesis that 6Ckine acts through CCR7 in bone marrow-derived dendritic cells.

Chemokine responsiveness and chemokine receptor expression have often been studied using dendritic cells generated in vitro from precursors or isolated from blood or other tissue and expanded in vitro. However, chemokine receptor expression can be affected by culturing dendritic cells in the presence of cytokines (27, 28, 34). Alterations in chemokine receptor levels on cultured dendritic cells raised the possibility that our findings using bone marrow-derived dendritic cells might not extend to physiologically generated dendritic cells. On the contrary, we show evidence that not only in vitro-derived, but also in vivo-derived dendritic cells can respond to 6Ckine and MIP-3β. First, freshly isolated, uncultured lymph node dendritic cells responded to 6Ckine and MIP-3β in transwell chemotaxis assays. The percentage of lymph node dendritic cells that responded in these assays (~15–20% of the input cells) was lower than that observed for cultured bone marrow-derived dendritic cells. However, evidence suggests that the lymph node dendritic cell population is heterogeneous with respect to surface marker expression and possibly lineage (37, 38), so that it is possible that 6Ckine may have been active on a particular subset of lymph node dendritic cells, which were not distinguished in our assays. While bone marrow-derived dendritic cells may model a subset of in vivo-derived dendritic cells, a substantial proportion of lymph node dendritic cells exhibit a chemokine receptor profile that remains to be elucidated. Interestingly, lymph node dendritic cells (as well as total $CD4^+$ lymph node cells) responded better to 6Ckine than to MIP-3β. The basis for this difference, which was not observed with bone marrow-derived dendritic cells, is unclear and currently under investigation.

Further evidence that 6Ckine and MIP-3β could chemoattract in vivo-generated dendritic cells came from observations that incubation of mouse skin in either chemokine increased emigration of dendritic cells out of the skin. In sum, these data indicate that dendritic cells obtained from lymphoid as well as nonlymphoid tissues are responsive to 6Ckine and MIP-3β.

Priming of rare T cells specific for a given Ag takes place in secondary lymphoid tissues; dendritic cells are fundamental in presenting Ag to naïve T cells and initiating an immune response. A critical step in this process is the migration of Ag-loaded dendritic cells from nonlymphoid sites of Ag deposition into lymph nodes and Peyer's patches, a process likely to be guided by chemokines. Our findings show that 6Ckine and MIP-3β are potent chemoattractants for in vitro-expanded bone marrow-derived dendritic cells and in vivo-generated lymph node and skin dendritic cells. Expression of 6Ckine and MIP-3β is limited primarily to lymphoid organs; 6Ckine is also expressed in HEV and lymphatic endothelium. Taken together, the evidence prompts the hypothesis that 6Ckine and MIP-3β are involved in directing dendritic cell homing to, and within, lymphoid tissue in vivo. The ability of these chemokines to attract dendritic cells as well as naïve T cells (17, 35, 39) presents an intriguing model wherein both types of cells are recruited to the same site via the actions of common chemokines, thus promoting the likelihood of their encounter and an ensuing immune response.

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