The Journal of Immunology

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CUTTING EDGE

Cutting Edge: Selective Usage of Chemokine Receptors by Plasmacytoid Dendritic Cells[1]

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The existence of dendritic cell (DC) subsets is firmly established, but their trafficking properties are virtually unknown. In this study, we show that myeloid (M-DCs) and plasmacytoid (P-DCs) DCs isolated from human blood differ widely in the capacity to migrate to chemotactic stimuli. The pattern of chemokine receptors expressed by blood M-DCs and P-DCs, with the exception of CCR7, is similar. However, most chemokine receptors of P-DCs, in particular those specific for inflammatory chemokines and classical chemotactic agonists, are not functional in circulating cells. Following maturation induced by CD40 ligation, the receptors for inflammatory chemokines are down-regulated, and CCR7 on P-DCs becomes coupled to migration. The drastically impaired capacity of blood P-DCs to migrate in response to inflammatory chemotactic signals contrasts with the response to lymph node-homing chemokines, indicating a propensity to migrate to secondary lymphoid organs rather than to sites of inflammation. *The Journal of Immunology,* 2001, 167: 1862–1866.

D endritic cells (DCs),[3] a highly specialized APC system critical for the initiation of CD4$^+$ T cell responses, are present, in different stages of maturation, in the circulation as well as in lymphoid and nonlymphoid organs (1). DCs are heterogeneous in terms of origin, morphology, phenotype, and function (2, 3). In the mouse thymus, two distinct subsets have been identified based on CD8α expression (4). CD8α^+ DCs seem to derive from an early thymic lymphoid precursor, suggesting a lymphoid origin, whereas CD8α^- DCs appear to be of myeloid origin (5). The existence of mouse DC subsets prompted the search for similar subpopulations in humans. Two distinct DC subsets

were originally defined in the human blood based on the expression of CD11c (6). More recent work has characterized these two subsets as belonging to the myeloid or lymphoid lineage and, although different denominations have been used, they can be defined as myeloid DCs (M-DCs) and plasmacytoid DCs (P-DCs) (7). M-DCs are characterized by a monocytic morphology, express myeloid markers like CD13 and CD33, the β_2 integrin CD11c, the inhibitory receptor Tg-like transcript 1 (ILT1) and low levels of the IL-3R α-chain CD123 (8). Conversely, P-DCs have a morphology resembling plasma cells, are devoid of myeloid markers, and express high levels of CD4, CD62 ligand (L), and CD123 (9, 10). M-DCs produce high levels of IL-12 (11), whereas P-DCs produce high levels of IFN-α (10, 12). Based on their capacity to induce, under appropriate conditions, predominantly Th1 or Th2 cells, human M-DCs and P-DCs have also been designated as DC1 and DC2 (11). In vivo studies indicate that P-DCs, in normal conditions, are preferentially localized in secondary lymphoid tissues, but their location in inflammatory conditions is still unclear (8, 10, 13).

The proper localization of DCs in secondary lymphoid organs and their recruitment at sites of inflammation in response to chemotactic stimuli are critical for an optimal immune response. In this study, we report that, despite a similar expression and modulation of chemokine receptors, circulating P-DCs, in contrast to M-DCs, fail to migrate in response to inflammatory chemokines, whereas both subsets respond to lymph node-homing chemokines following CD40 ligation. These different migration programs underscore the distinct lineage of these DC subsets and point to their distinct roles in the induction and regulation of the immune response.

Materials and Methods

Peripheral blood DC purification

PBMC were isolated from buffy coats by Ficoll gradient (Pharmacia Biotech, Uppsala, Sweden) and peripheral blood DCs were purified with a blood DC cell isolation kit (Miltenyi Biotec, Bergish Gladblach, Germany) to a purity of 80–90%. Purified blood DCs were stained with Lin 1-FITC and HLA-DR PerCP, followed by CD123-PE or CD11c-PE and sorted with a FACSVantage (BD Biosciences, Mountain View, CA). Alternatively, blood M-DCs and P-DCs were magnetically sorted with BDCA-1 and BDCA-4 cell isolation kits (Miltenyi Biotec) (14), respectively, to a purity of 90–98% in both cases.

Flow cytometric analysis

Flow cytometric analysis was performed as previously described (15) in the presence of 100 μg/ml mouse IgG using the following mAbs from BD PharMingen (San Diego, CA): anti-lineage mixture 1 (containing mAbs specific for CD3, CD14, CD16, CD19, CD20, and CD56) FITC, anti-HLA-DR Cy/PerCP, anti-CD123-PE, anti-CD11c-PE, anti-CD62L-FITC, anti-CD36-FITC, anti-CD45RA-FITC, anti-CCR4, anti-CCR5-PE, anti-CCR7, anti-CXCR1-PE, anti-CXCR2-PE, and anti-CXCR5-PE. Anti-CCR1-PE, anti-CCR2-PE, anti-CCR6-PE, anti-CXCR3-FITC, and anti-CXCR4-FITC were

[3] Abbreviations used in this paper: DC, dendritic cell; M-DC, myeloid DC; P-DC, plasmacytoid DC; L, ligand; MCP-1, monocyte chemoattractant protein 1; MIP-1, macrophage inflammatory protein 1; IP-10, inflammatory protein 10; PAF, platelet-activating factor; ILT1, Ig-like transcription factor 1; SDF-1, stromal-derived factor 1; I-TAC, IFN-inducible T cell α chemoattractant; PARC, pulmonary and activation-regulated chemokine; Mig, monokine induced by IFN-γ; CCL, CC chemokine ligand; ELC, EBI 1 ligand chemokine.

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purchased from R&D Systems (Minneapolis, MN). The anti-ILT1 mAb, a kind gift from Dr. M. Colonna (Basel Institute of Immunology, Basel, Switzerland), has been described previously (16). The anti-CCR3 mAb was a kind gift from Dr. C. McKay (Millennium Pharmaceuticals, Cambridge, MA). The fMLP receptor was revealed using fMLP-FITC (Molecular Probes, Eugene, OR). Cells were analyzed with a FACScan flow cytometer using CellQuest software (BD Biosciences).

Chemotaxis assay

DC subset migration was measured by chemotaxis through a 5-μm pore polycarbonate filter in 24-well transwell chambers (Corning, Cambridge, MA). Enriched blood DCs were stained to distinguish Lin1$^-$HLA-DR$^+$CD11c$^+$ cells (M-DCs) and Lin1$^-$HLA-DR$^+$CD11c$^-$ cells (P-DCs). Alternatively, sorted DC subsets were used, as specified in the text. Serial dilutions of chemokines were added to the lower wells and 10^5 cells to the transwell insert. The following chemokines were used: RANTES, stromal-derived factor 1 (SDF-1), eotaxin, macrophage-derived chemokine, I-309, IFN-inducible T cell α chemoattractant (I-TAC), pulmonary and activation-regulated chemokine (Dictagene, Geneva, Switzerland), monocyte chemoattractant protein 1 (MCP-1) (BD PharMingen), macrophage inflammatory protein 3α (MIP-3α), secondary lymphoid tissue chemokine, thymus-expressed chemokine, IFN-γ-inducible protein of 10 kDa (IP-10), monokine induced by IFN-γ (Mig), B lymphocyte chemokine, and IL-8 (R&D Systems). In addition, the chemotactic stimuli platelet-activating factor (PAF) and fMLP (Sigma, St. Louis, MO) were used. After incubation for 90 min at 37°C, the migrated cells were analyzed with a FACScan flow cytometer using CellQuest software (BD Biosciences). The number of cells in the starting population and the migrated population was calculated for each phenotype, and the percent migration was determined from these values. Each experiment was performed in triplicate.

Intracellular calcium fluxes

Magnetically sorted M-DCs and P-DCs (10^6 cells/ml) were loaded with 4 μM fluo-3-acetoxymethyl ester in the presence of 1 μM pluronic F-127 (Molecular Probes) in HBSS containing 5% FCS for 30 min at 37°C. Cells were washed twice, incubated with the indicated chemokine concentrations or with 1 μg/ml ionomycin, and analyzed with a FACScan.

Results

Similar chemokine receptor expression but distinct migration patterns of blood M-DCs and P-DCs

Enriched peripheral blood DCs (Lin$^-$ CD4$^+$) from healthy donors were sorted based on the expression of CD123. Two discrete populations, both HLA-DR$^+$, were obtained, expressing high and low levels of CD123 (Fig. 1*A*). The CD123^{low} cells expressed the ILT1 (16), whereas the CD123^{high} were ILT1$^-$. Based on the previously described subsets of blood DCs (10), the CD123^{low}ILT1$^+$ cells correspond to M-DCs and the CD123^{high}ILT1$^-$ cells to P-DCs. Additional phenotypic analysis of the sorted populations demonstrated that M-DCs were CD11c$^+$, CD62L^{low}, CD36^{low}, and CD45RA^{low}, whereas P-DCs were CD11c$^-$, CD62L^{high}, CD36^{high}, and CD45RA^{high} (data not shown), confirming the subset assignment (10).

The expression of chemokine receptors on sorted blood M-DCs and P-DCs was, in general, fairly similar (Fig. 1*B*). Both subsets expressed relatively high levels of CCR2, CCR5, and CXCR4. CCR4 and CXCR2 were very weakly expressed on both M-DCs and P-DCs. CCR6, CXCR1, and CXCR5 were not expressed on either DC subset. CCR1 was not expressed at all on M-DCs and very weakly on P-DCs. CCR7 and CXCR3 expression diverged. CCR7 expression was negligible on blood M-DCs but very high on P-DCs, whereas CXCR3 expression was weak on M-DCs and relatively high on P-DCs. The receptor for fMLP was higher on M-DCs than on P-DCs.

In contrast with the overall similar pattern of chemokine receptor expression, with the exception of CCR7, circulating M-DCs and P-DCs exhibited a profoundly different migration capacity in response to chemokines (Fig. 2). A relatively high proportion of M-DCs migrated in response to RANTES, renamed CCL5 (17), MCP-1/CCL2, and SDF-1/CXCL12, whereas a lower proportion
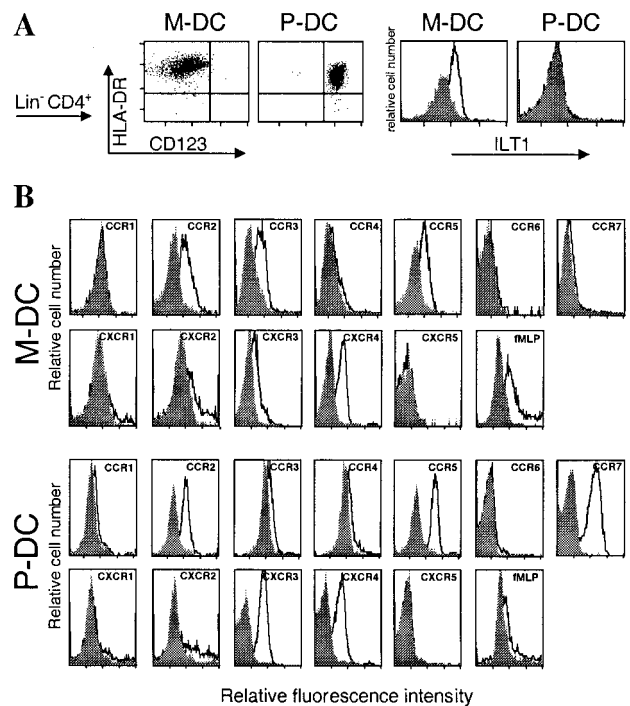
FIGURE 1. Similar chemokine receptor expression in blood DC subsets. *A*, Phenotype of M-DCs and P-DCs sorted for CD123 expression from Lin-1$^-$HLA-DR$^+$ human PBMC. ILT1 is selectively expressed on M-DCs. Isotype controls are in gray. *B*, Chemokine receptor expression analyzed on sorted M-DCs and P-DCs directly after isolation. Isotype controls are shown in gray. Results are from a representative experiment of two to five performed.

migrated in response to eotaxin/CCL11, MIP-3α/CCL20, IP-10/CXCL10, I-TAC/CXCL11, and Mig/CXCL9. No significant migration was seen in response to MDC/CC chemokine ligand (CCL)22, thymus and activation-regulated chemokine/CCL17, EBI 1 ligand chemokine (ELC)/CCL19, I-309/CCL1, thymus-expressed chemokine/CCL25, B lymphocyte chemokine/CXCL13, and IL-8/CXCL8. Conversely, P-DCs migrated only in response to SDF-1, the ligand of CXCR4. SDF-1 has previously been reported to induce migration of both P-DCs and M-DCs (18). The migration of P-DCs to SDF-1, although lower than M-DCs, was substantial (percentage of migrated cells 6.4 $\pm$ 1.6 vs 33.2 $\pm$ 3.6, $n = 9$). The migration index to SDF-1 was 249 $\pm$ 52 for P-DCs and 42 $\pm$ 9 for M-DCs, arguing against a generalized migration defect in P-DCs. Intriguingly, in addition to CXCR4, P-DCs expressed CCR7 and CXCR3, but did not migrate in response to any of the ligands of these two receptors, except for a negligible migration (<2% of input cells) to the highest dose of ELC tested (1 μg/ml). Similarly, the high expression of CCR2 and CCR5 by P-DCs was not paralleled by migration in response to MCP-1 or RANTES. Thus, although blood P-DCs can migrate in response to chemokines, as shown by the chemotactic response to SDF-1, it appears that most of the chemokine receptors expressed by this DC subset are not functional. This conclusion is also supported by the fact that a calcium flux was observed following stimulation of P-DCs with SDF-1 but not IP-10 nor RANTES, indicating a proximal defect in ligand signaling via CXCR3 and CCR5, respectively (Fig. 3).

The chemokine receptors expressed by M-DCs purified simultaneously with the P-DCs from the same blood donors were instead all functional, as demonstrated by migration of M-DCs in
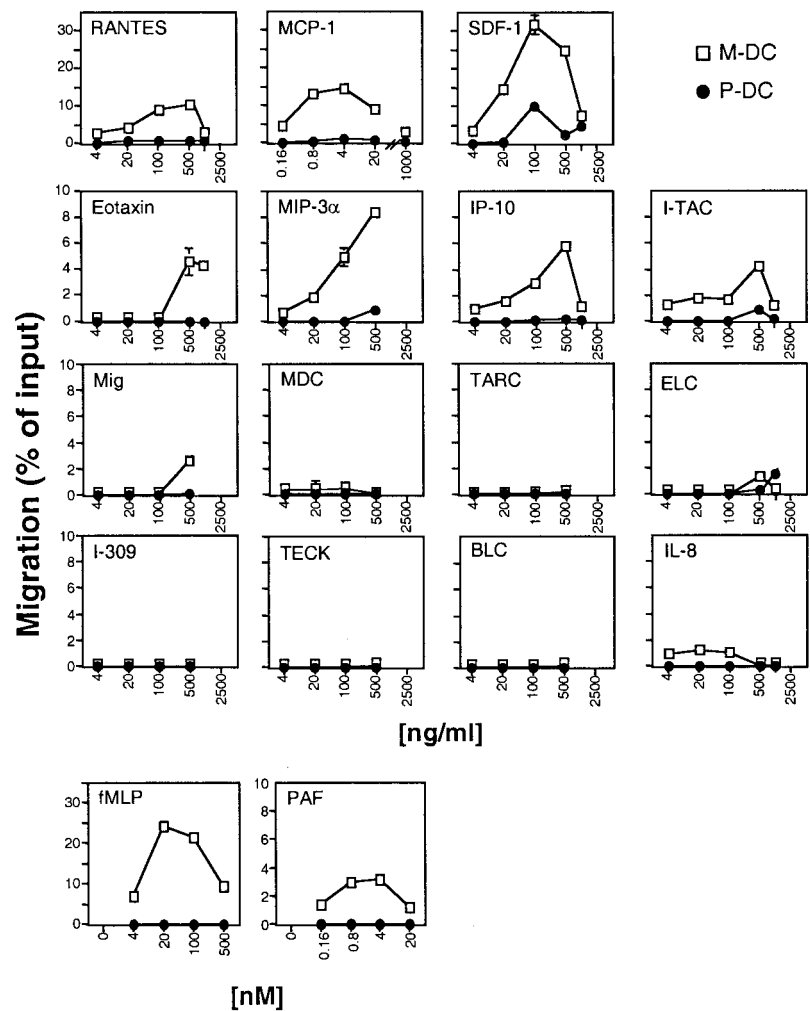
FIGURE 2. Migration of blood M-DCs and P-DCs to chemotactic stimuli. Enriched blood DCs were stained to allow the identification of M-DCs and P-DCs, and their migration (10^5 input cells/well) to different concentrations of chemotactic stimuli was measured. The average absolute numbers of M-DC and P-DC input cells/well were 16 ± 7 and $47 \pm 18 \times 10^3$. Results are expressed as mean percent migration of input cells $\pm$ SE from triplicate wells. Representative experiments of three to five performed are shown.

response to the respective ligands. Both M-DCs and P-DCs expressed also intermediate levels of a "classical" chemotactic receptor, specific for formylated bacterial peptides (fMLPR). However, only M-DCs migrated in response to formyl-methionyl-leucyl-phenylalanine receptor. In addition, PAF, a bioactive lipid with chemotactic properties, induced migration of M-DCs but not P-DCs (Fig. 2). Based on these results, circulating P-DCs would not be expected to migrate in response to inflammatory stimuli.

Modulation of responsiveness to chemokines in mature M-DCs and P-DCs

Signals that induce DC maturation affect also their capacity to migrate in response to chemotactic signals (19–21). We have examined the migration to chemokines and the expression of chemokine receptors in M-DCs and P-DCs ex vivo and after maturation induced by CD40 ligation, as shown by CD83 expression (Fig. 4). Culture of M-DCs and P-DCs with CD40L-expressing cells abrogated the capacity of freshly isolated M-DCs to migrate to RANTES and IP-10, while up-regulating the capacity to migrate in response to ELC and maintaining responsiveness to SDF-1, despite undetectable CXCR4 expression. As with circulating M-DCs, the expression of chemokine receptors in cells matured in vitro paralleled their capacity to migrate in response to chemokines. In contrast, P-DCs cultured for 2 day in IL-3 and stimulated by CD40 ligation lost the capacity to respond to SDF-1, the only chemotactic signal tested active on blood P-DCs, but acquired the capacity to migrate in response to ELC.
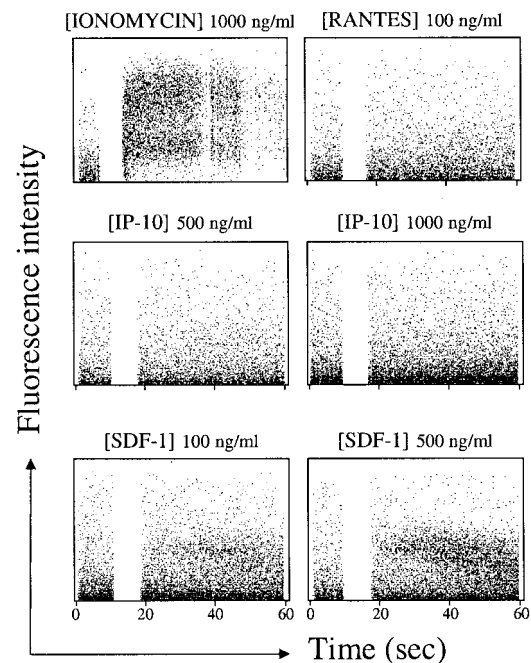
FIGURE 3. Intracellular calcium fluxes induced by chemokines in P-DCs. Calcium fluxes were measured as described in *Materials and Methods* in the presence of ionomycin (1 μg/ml), RANTES (100 ng/ml), IP-10 (500 and 1000 ng/ml), and SDF-1 (100 and 500 ng/ml).

FIGURE 4. Coupling of CCR7 in mature P-DCs. Magnetically purified M-DCs and P-DCs (5×10^4 cells/well) were analyzed for migration to the indicated chemokines (100 ng/ml for RANTES and SDF-1, 500 ng/ml for ELC and IP-10) and expression of chemokine receptors directly after isolation or after 48 h culture with GM-CSF/IL-4 or IL-3, respectively, in the presence of CD40L. Migration is expressed as in Fig. 2. Chemokine receptor expression is shown as Δ geometric mean fluorescence intensity (MFI), with isotype control subtracted. CD83 expression was simultaneously analyzed and isotype controls are shown in gray. Results are from a representative experiment of two to four performed.
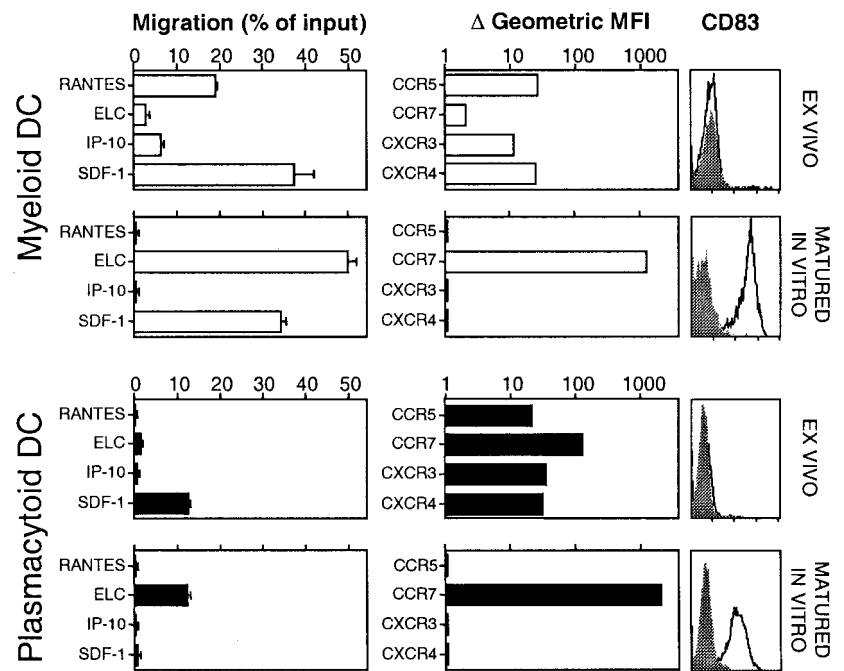
Unlike blood P-DCs, the expression of chemokine receptors in mature P-DCs, with no detectable CCR5, CXCR3, and CXCR4 but high expression of CCR7, paralleled their capacity to migrate in response to chemokines. This indicates that following maturation, expression of CCR7 by P-DCs becomes functionally coupled with migration in response to ELC. CCR7 was already expressed at high levels by blood P-DCs, suggesting that in this DC subset maturation is associated to a functional coupling of this receptor rather than with gene transcription, as observed in monocyte-derived DCs (19, 20).

In both subsets, the modulation of chemokine receptor expression upon maturation followed the pattern exhibited by monocyte-derived DCs, with down-regulation of CCR5, CXCR3, and CXCR4 and strong up-regulation of CCR7 (19). Thus, the expression of chemokine receptors not functional in blood P-DCs, like CCR5, CCR7, and CXCR3, is modulated similarly to their functional counterparts expressed by M-DCs.

Discussion

Our results show that M-DCs and P-DCs circulating in the human blood have distinct migration patterns. Although M-DCs could migrate in response to several constitutive and inducible chemokines, freshly isolated P-DCs showed responsiveness only to SDF-1. Despite their divergent capacity to migrate, P-DCs and M-DCs expressed a comparable repertoire of adhesion molecules (e.g., CD50, CD58, CD62L, CLA; data not shown), and chemokine receptors that were, however, not functional in P-DCs.

P-DCs are found in lymph nodes where they have migrated from the blood via the high endothelial venules (13), but this does not appear to be mediated by responsiveness to IP-10, Mig, or I-TAC, the ligands of CXCR3. Indeed, blood P-DCs fail to migrate in response to these chemokines, although this was inferred by their expression of CXCR3 (10). Rather P-DCs may reach lymph nodes, independently of inflammatory stimuli (8), using CXCR4, the receptor of SDF-1, a chemokine expressed within the lymph node (22), and CD62L that interacts with L-selectin ligands expressed by high endothelial venules (23). This interaction may provide a maturation signal (24) that couples CCR7 with migration, allowing proper positioning of P-DCs in the T cell areas of secondary lym-

phoid tissues in response to secondary lymphoid tissue chemokine and ELC. A comparative analysis of migration and chemokine receptor expression in blood and tonsil or lymph node P-DCs is ongoing to further clarify this issue.

The uncoupling of chemokine receptor expression and migration has been reported in other cell types. Germinal center B lymphocytes express CXCR4 but do not migrate to SDF-1 (25), and circulating B cells do not respond to MIP-3α despite the surface expression of CCR6 (26). Furthermore, the expression of nonfunctional IL-8 receptors has been described in human monocytes (27) and monocyte-derived DCs (28). Finally, uncoupled chemokine receptors induced by IL-10 in LPS-stimulated monocyte-derived DCs have been proposed to generate functional decoy receptors that act as molecular sinks and scavengers for inflammatory chemokines (29). Although blood P-DCs constitute a sparse population that may not accumulate at sites where these ligands are active, it is possible that the membrane expression of nonfunctional chemokine receptors by P-DCs may influence the trafficking properties of activated cells and contribute to the regulation of the immune response.

During maturation of human monocyte-derived DCs, the expression of CCR1, CCR2, CCR5, CCR6, and CXCR1 is down-regulated with increased expression of CCR7 (19, 20, 30). This "switch paradigm" seems to hold also for M-DCs and P-DCs. However, blood P-DCs already express high membrane levels of CCR7, and this expression is further increased during maturation. Intriguingly, maturation allows coupling of CCR7 to cell migration in P-DCs. This is associated with down-regulation of receptors for inflammatory chemokines, indicating that also mature P-DCs fail to respond to inflammatory chemotactic stimuli. In addition to maturation, it is possible that other signals, for example, via Toll receptors (31) or virus infection (32), may allow rapid coupling of migration with chemokine receptor expression. This may endow P-DCs with the capacity to migrate to sites of inflammation, as shown by their dramatic increase in the nasal mucosa during the allergic reaction (33). Migration of M-DCs and P-DCs to SDF-1 is also differently regulated by maturation, being maintained in the former and lost in the latter cell subset. Therefore, M-DCs and

P-DCs present a different regulation of their migration profile, both at the immature and mature stage.

In conclusion, the different migration programs of blood M-DCs and P-DCs, and the expression by P-DCs of nonfunctional chemokine receptors point to a primary involvement of M-DCs in inflammation and of P-DCs in the homeostatic control of the immune response. This would be consistent with the capacity of P-DCs not only to induce Th1 (34) but also Th2 (11) and possibly T regulatory (35) cells.

Acknowledgments

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