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Receptor Expression and Responsiveness of Human Dendritic Cells to a Defined Set of CC and CXC Chemokines[1]

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Dendritic cells (DC) are migratory cells that exhibit complex trafficking properties in vivo. The present study was designed to characterize receptor expression and responsiveness to chemoattractants of human DC obtained from PBMC by culture with granulocyte/macrophage-CSF and IL-13. DC expressed appreciable levels of the CCR1, CCR2, and CCR5 receptors for the CC chemokines and the chemokine receptors CXCR1, CXCR2, and CXCR4. DC increased intracellular free calcium and migrated in response to the CC chemokines MCP-3, MCP-4, RANTES, MIP-1α, MIP-1β, and MIP-5/HCC2 and the CXC chemokine SDF-1. In contrast, the CC chemokines MCP-1 and eotaxin had little or no activity in the concentration range tested (up to 1 μg/ml). IL-8 and Gro-β (CXC) and lymphotactin (C chemokines) were also inactive. DC did not respond to 5-HETE, whereas platelet-activating factor was an active agonist. Selected chemokines active on DC in terms of migration and calcium fluxes were examined for their capacity to modulate endocytosis and Ag presentation. Under conditions in which TNF-α was active, MCP-1, MCP-3, MIP-1α, and RANTES did not affect these two responses. Thus, among hemopoietic elements, DC respond to a unique set of CC and CXC chemokines, and their responsiveness is restricted to migration with no effect on Ag capture and presentation. Chemokines may play a role in the trafficking of DC under resting or stimulated conditions. Chemokine receptors expressed in DC are likely to underlie HIV infection of this cell type. *The Journal of Immunology,* **1997, 159: 1993–2000.**

Dendritic cells (DC)[3] are bone marrow-derived leukocytes specializing in Ag uptake, processing, and presentation to T lymphocytes. DC are most potent among APCs and are believed to be indispensable to the initiation of a primary immune response (1, 2). For their central role in the regulation of immunity, DC are considered interesting tools and targets for immunotherapeutic interventions (3, 4).

DC progenitors from the bone marrow enter the blood and seed nonlymphoid tissues, where they develop into immature DC, with high ability in Ag uptake and processing, and yet low ability in T cell stimulation. DC are localized in the epithelia, such as skin epidermis (Langherans cells), the gastrointestinal and genito-urinary tracts, airways, and in the interstitial spaces of many solid organs (heart, liver, kidney) (1, 5, 6). Locally produced inflammatory cytokines (e.g., TNF and IL-1) and the encounter with an Ag promote the maturation and migration of DC to regional lymph nodes via afferent lymphatics and/or blood (6–8). These migratory cells undergo maturation from a "processing" to a "presenting" functional phenotype, characterized by the expression of costimulatory molecules, cytokine production, and high ability to stimulate T cell proliferation (2, 8, 9). Thus, migration is required for the accomplishment of DC functional activity.

Information on the signals involved in the recruitment of DC into tissues is sparse. Intradermal administration of granulocyte/macrophage (GM)-CSF leads to an increased number of DC within the human dermis (10). TNF, and possibly other LPS-induced cytokines, quickly recruit DC in the airway epithelia in a model of respiratory infection (11), and systemic administration of LPS induces a profound loss of MHC class II^+ cells from heart and kidney in the mouse (9).

Recently, it was found that "classical" chemotactic agonists, such as formylated peptides and C5a, as well as some C-C chemokines (RANTES, MIP-1α, and MCP-3), induce directional migration of monocyte-derived DC, $CD34^+$ cell-derived DC, and Langerhan's cells in vitro (12–14). Chemokines are a growing superfamily of low m.w. chemotactic proteins that can be divided into four branches according to the position of the first cysteine pair (C-X-C or α and C-C or β families), the lack of two of the four cysteines (C or γ), or the presence of three spacing amino acids in the first cystein tandem (CX3C or δ) (15–19). C-X-C chemokines, of which IL-8 is the prototype, are mainly active on neutrophils and T lymphocytes. C-C chemokines have a wider spectrum of action, being active on monocytes, granulocytes, T and B lymphocytes, NK cells, and DC (15–18). Lymphotactin, the only C chemokine so far described, is active on T lymphocytes and NK cells (20, 21). Chemokines are produced by multiple cell types, including monocyte/macrophages, endothelial cells, mesothelial cells, fibroblasts,

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[3] Abbreviations used in this paper: DC, dendritic cells; GM, granulocyte/macrophage; MCP, monocyte chemotactic protein; PAF, platelet activating factor; $[Ca^{2+}]_i$, intracellular free calcium concentration; MDC, macrophage-derived chemokine; MIP, macrophage inflammatory protein; IC_{50}, 50% inhibitory concentration; PTox, Bordetella pertussis toxin; 5-HETE, 5-hydroxy-6,8,11,14,eicosatetranoic acid.

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keratinocytes, and lymphocytes, and bind to seven transmembrane domain, G protein-coupled receptors (15–18, 22, 23). Four receptors for C-X-C chemokines (CXCR1 to 4) and five for C-C chemokines (CCR1 to 5) were recently cloned. These receptors show a promiscuous pattern of ligand recognition and are differentially expressed and regulated in leukocytes (23–33).

The goals of the present study were 1) to identify the chemokine receptors present on DC; 2) to extend the preliminary characterization of chemokines active on DC to the new, recently identified C-C chemokines and to proteins of the C-X-C and C families; and 3) to investigate whether active chemokines can modulate functional responses other than chemotaxis in DC. The data presented here show that in vitro-differentiated, monocyte-derived DC express both C-X-C and C-C chemokine receptors and migrate to a selected pattern of chemokines when compared with other leukocyte populations (e.g., monocytes and T lymphocytes). In addition, two characteristic functions of DC, endocytosis of macromolecules and induction of proliferation of allogenic T cells, were not affected by active C-C chemokines, suggesting that these proteins act on DC mainly as migratory signals.

Materials and Methods

Cytokines

Human rMCP-1, lymphotactin, MIP-1β, and eotaxin were from PeproTech Inc. (Rocky Hill, NJ). Human rIL-8 was from Dainippon (Osaka, Japan). Human rMCP-3 and IL-13 were a kind gift from Dr. A. Minty (Sanofi Elf Bio Recherches, Labège, France). Human rMIP-1α/LD78 was from Dr. L. Czaplewski (British Bio-technology Limited, Cowley, U.K.), and human SDF-1 was from R&D System (Minneapolis, MN). MCP-4 (34) was expressed in COS cells as previously described (35). RANTES and MIP-5/HCC2 were chemically synthesized (36). MIP-5/HCC2 is a novel human CC chemokine that shows high sequence identity to MIP-3 (76.7%), MIP-4 (63.2%), MIP-1α (75.4%), and MIP-1β (66.7%).[4] Both the nucleotide and protein sequence are deposited in the GenBank database (accession no. Z70292) and in the SwissProt database (accession no. Q16663), respectively. Human recombinant GM-CSF and TNF-α were a generous gift from Sandoz (Basel, Switzerland), and BASF (Knoll, Germany), respectively. Cytokines were endotoxin free as assessed by *Limulus* amebocyte assay. FMLP and platelet-activating factor (PAF) were from Sigma Chemical Co. (St. Louis, MO).

DC culture

Highly enriched blood monocytes (>95% $CD14^+$) were obtained and purified from buffy coats (through the courtesy of Centro Trasfusionale, Ospedale Sacco, Milan, Italy) by Ficoll and Percoll gradients and purified by panning on CD6-coated plastic dishes as described (12, 37). Monocytes were cultured for 7 days at 1×10^6/ml in six-well multiwell tissue culture plates (Falcon; Becton Dickinson, Lincoln Park, NJ) in RPMI 1640 (Biochrom, Berlin, Germany), 10% FCS (HyClone, Logan, UT) supplemented with 50 ng/ml GM-CSF, and 10 ng/ml IL-13. We previously demonstrated that monocyte-derived DC generated in the presence of GM-CSF + IL-13 are morphologically and functionally identical to DC cultured with GM-CSF + IL-4 (12, 37). These cells were >80% $CD1a^+$, >90% MHC class II^+, <10% $CD14^+$, <2% $CD3^+$, and <4% $CD20^+$. For some experiments, cells were further depleted of $CD14^+$ cells (<1% $CD14^+$) by CD-14-coated Dynabeads (Unypath, Milan, Italy).

Northern blot analysis

DC were prepared as described above, and total RNA was extracted by the guanidinium thiocyanate method, blotted, and hybridized as described (33). Probes were labeled by the Megaprime DNA labeling system (Amersham, Buckinghamshire, U.K.) with [α-^{32}P]dCTP (3000 Ci/mmol, Amersham). Membranes were prehybridized at 42°C in Hybrisol (Oncor, Inc., Gaithersburg, MD) and hybridized overnight with 1×10^6 cpm/ml of ^{32}P-labeled probe. Membranes were then washed three times with 2× SSC (1× SSC = 0.15 M NaCl, 0.015 M sodium citrate, pH 7.0), 0.1% SDS at room temperature for 10 min, twice with 2× SSC, 1% SDS at 60°C for 20 min, and then with 0.1× SSC for 5 min, before being autoradiographed using Kodak XAR-5 films and intensifier screens at −80°C. CCR2B cDNA was obtained by PCR amplification of the reported sequence (33, 38). CCR3, CCR4, CCR5, CXCR1, and CXCR3 cDNAs were obtained as previously described (25). CCR1 and CXCR2 cDNAs (39) were kindly donated by Dr. Ji Ming Wang (Science Applications International Corp. Frederick, National Cancer Institute, Frederick, MD). CXCR4 cDNA was provided by Dr. Marc Parmentier (IRIBHN, Bruxelles, Belgium).

Receptor binding assays

Competition for the binding of ^{125}I-labeled MCP-3 ([^{125}I]MCP-3; sp. act., 2200 Ci/mmol; DuPont de Nemours, Dreieich, Germany), [^{125}I]MCP-3, and ^{125}I-labeled IL-8 ([^{125}I]IL-8; sp. act., 2000 Ci/mmol; Amersham) to DC was conducted as described previously (40). DC (1×10^6/200 μl) in binding medium (RPMI 1640 with 10 mg/ml BSA, Sigma Chemical Co., Milan, Italy) were incubated with 0.5 nM labeled chemokine in the presence of different concentrations of unlabeled cytokines at 4°C for 2 h. At the end of the incubation, cells were pelleted through a cushion of silicon oil by microcentrifugation. The radioactivity present in the tip of the tubes and in the supernatants was evaluated using a gamma counter.

Migration assay

Cell migration was evaluated using a chemotaxis microchamber technique as previously described (12). Twenty-seven microliters of chemoattractant solution or control medium (RPMI 1640 with 1% FCS) were added to the lower wells of a chemotaxis chamber (Neuroprobe, Pleasanton, CA). A polycarbonate filter (5-μm pore size; Neuroprobe) was layered onto the wells and covered with a silicon gasket and with the top plate. Fifty microliters of cell suspension ($0.7–1 \times 10^6$/ml) were seeded in the upper chamber. The chamber was incubated at 37°C in humidified air in the presence of 5% CO_2 for 90 min. At the end of the incubation, filters were removed, stained with Diff-Quik (Baxter s.p.a., Rome, Italy) and five high power oil-immersion fields (100×) were counted. Results are expressed as the mean number of migrated cells in 10 high power fields. Each experiment was performed in triplicate.

Measurement of intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$)

Changes in $[Ca^{2+}]_i$ were monitored using the fluorescent probe fura-2 as previously described (40) according to the technique reported by Grynkiewicz et al. (41). Briefly, DC (10^7/ml) were resuspended in RPMI 1640 and incubated with 1 μM fura-2 acetoxymethyl ester (Calbiochem, San Diego, CA) at 37°C for 20 min. After incubation, cells were washed and resuspended in HBSS (Biochrom) containing 1.2 mM $CaCl_2$ and kept at room temperature until used. Fura-2 fluorescence was measured in a Perkin-Elmer LS 50B spectrophotometer (Perkin-Elmer Instruments, Norwalk, CT) at 37°C with cells ($3–5 \times 10^6$/ml) continuously stirred. Samples were excited at 340 and 380 nm, and emission was continuously recorded at 487 nm.

Flow cytometric analysis of CXCR1 and CXCR2 expression

Anti-CXCR1 (5A12–5) and -CXCR2 (6C6–1C) (42) were kindly provided by Dr. C. R. Mackay (LeukoSite, Inc. Cambridge, MA). DC were incubated with saturating amounts of mAbs and, after washing with saline, with fluorescein-conjugated $F(ab')_2$ goat anti-mouse Ig (Techno Genetics, Turin, Italy). Analysis of fluorescence was performed by a FACStar^{Plus} calibrated with Calibrite beads (Becton Dickinson) (43).

Mixed leukocyte reaction

DC were added in graded doses to 1×10^5 irradiated (3000 rad) purified allogenic T cells in 96-well flat-bottom microtest plates. Responder cells were cord blood T cell depleted of autologous APC by passage with CD14- and CD19-coated Dynabeads (37). Each experimental group was tested in triplicate. [^{3}H]Thymidine (5Ci/μmol; Amersham) incorporation was measured on day 5 after a 16-h pulse.

Endocytosis of FITC-dextran

Endocytosis was measured as the cellular uptake of FITC-dextran and quantitate by flow cytometry (37). Approximatively 2×10^5 cells for each sample were incubated in the presence of 1 mg/ml of FITC-dextran (m.w. 70,000; Sigma Chemical Co., St. Louis, MO). After incubation, cells were washed twice with PBS and fixed with 1% formalin. FITC-dextran uptake of at least 8000 cells was evaluated by FACS analysis.

[4] F. Coulin, C. A. Power, S. Alouani, M. C. Peitsch, J.-M. Schroeder, M. Moshizuki, I. Clark Lewis, and T. N. C. Wells. Characterization of MIP-5/HCC2: a new member of the MIP family of chemokines. *Submitted for publication.*

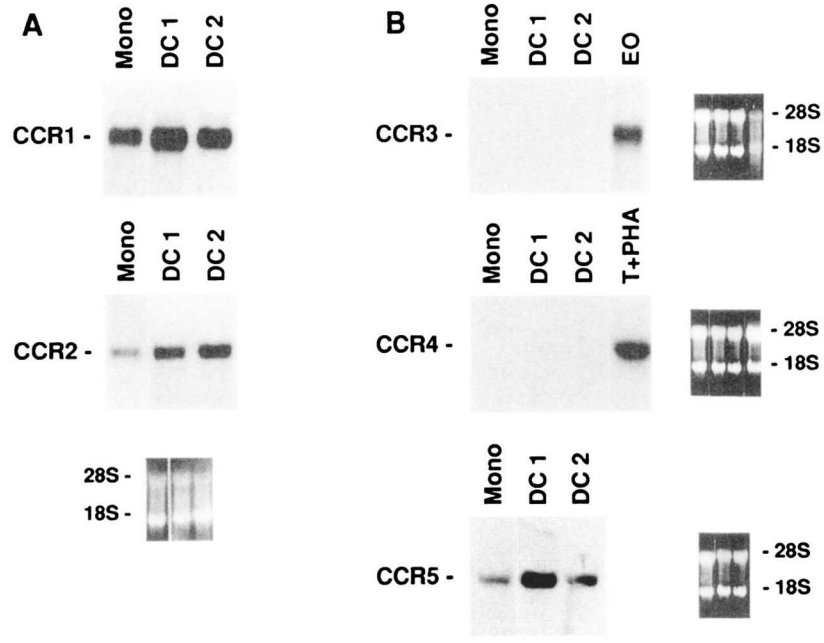

FIGURE 1. Expression of CC chemokine receptors in DC. Fifteen micrograms of total RNA was purified from DC or fresh human monocytes and used in Northern blot analysis. Filters were probed for the expression of CCR1 and CCR2 (*A*) and CCR3, CCR4, and CCR5 (*B*). Results of two independent cultures of DC are shown and are representative of at least four different cell preparations. The autoradiographs were obtained after a 6-h exposure.

Statistical analysis

Results are presented as mean (±SD) of a representative experiment or as mean (±SE) of several experiments. Data were analyzed by Student's *t* test.

Results

Expression of chemokine receptors in DC

A first objective of the present study was to obtain a comprehensive characterization of the pattern of CC and CXC chemokine receptors expressed by DC. Figure 1 shows that, in a manner similar to monocytes, DC express the transcripts for CCR1, CCR2, and CCR5. In our experimental conditions, only a faint signal was detectable for CCR3 and CCR4. On the contrary, CCR3 and CCR4 mRNAs were easily detectable in eosinophils and PHA-activated T lymphocytes, respectively. DC also express detectable levels of mRNA for CXCR1 and CXCR2 (IL-8 receptors A and B, respectively) and for CXCR4 (formerly LESTR or fusin). No expression of CXCR3 was detected (Fig. 2). It is interesting to note that although DC express the transcripts for MCP-1 receptor (CCR2) and IL-8 receptors, they do not functionally respond to these chemokines (Ref. 12 and Table I).

DC migration in response to C-C chemokines

The ability of DC to migrate in response to a variety of chemotactic signals was investigated using a micromultiwell chemotaxis chamber assay. Table I summarizes the results obtained using classical chemotactic agonists, C-X-C, C-C, and C chemokines, cytokines, and bioactive lipids. In addition to C5a, FMLP, MCP-3, RANTES, and MIP-1α, previously shown to be potent DC chemoattractants (12–14), MIP-1β, MIP-5/HCC2, and MCP-4, three recently characterized C-C chemokines, and SDF-1 (C-X-C) appeared to be the most active proteins among the stimuli tested (Fig. 3). The four agonists induced DC chemotaxis in a concentration-dependent manner. Peak activity for MIP-1β and MIP-5/HCC2 was observed at the concentration of 100 ng/ml, with a number of cell migrated that was 186 ± 20% and 170 ± 8 (n = 3) of that observed with 100 ng/ml of MCP-3, used as reference chemokine, respectively (Fig. 3*A*). DC migration to MCP-4 and to SDF-1 was slightly higher than that obtained with an optimal (100 ng/ml) concentration of MCP-3, although for SDF-1, a 1-log higher concentration was needed (Fig. 3, *B* and *C*). Monocytes and T lymphocytes were previously reported to migrate in response to SDF-1 in a similar concentration range (44–46). Eotaxin (C-C), lymphotactin (C), and all of the other C-X-C chemokines tested were inactive (Table I). 5-HETE, an arachidonic acid metabolite active on monocytes (47), was inactive; on the contrary, PAF, a weak chemotactic factor for phagocytic cells, showed a significant chemotactic activity for DC (Table I).

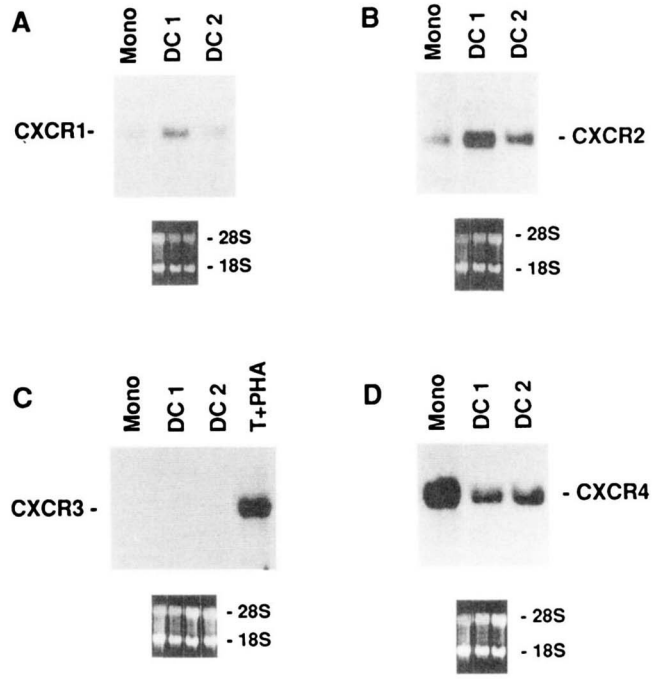

FIGURE 2. Expression of CXC chemokine receptors in DC. Fifteen micrograms of total RNA was purified from DC and used in Northern blot analysis. Results of two independent cultures of DC are shown and are representative of at least four different cell preparations. The autoradiographs were obtained after a 6-h exposure.

Table I. *Activity of Chemotactic Agonists on DC*[a]

Chemoattractants	Chemotaxis	Calcium Transients[b]
Classical		
C5a	82 ± 8 (5)[c]	++ (5)
fMLP	92 ± 5 (33)	+++ (30)
C-X-C Chemokines		
IL-8	1.5 ± 0.5 (5)	+ (3)
Gro-β	nd	− (1)
IP-10	1.4 (2)	nd
SDF-1	170 (2)	+++ (2)
C-C Chemokines		
MCP-1	9.0 ± 1.0 (31)	+ (5)
MCP-2	18 (2)	+ (2)
MCP-3	100 (37)	+++ (20)
MCP-4	145 ± 15 (3)	+ (2)
MIP-1α	100 ± 10 (18)	+++ (15)
MIP-1β	150 ± 20 (5)	++ (8)
MIP-5/HCC2	132 ± 10 (2)	+++ (5)
RANTES	95 ± 7 (13)	+++ (10)
Eotaxin	10 (2)	+ (2)
C Chemokines		
Lymphotactin	1 (2)	− (2)
Lipids		
PAF	+++ (5)	++ (2)
5-HETE	0.4 (2)	− (1)
Cytokines		
GM-CSF	0.5 (2)	nd
TNF	0.2 (1)	nd

[a] Chemokines were used at the concentration of 100 ng/ml with the exception of SDF-1 (1 μg/ml). Negative results were confirmed with higher concentrations (up to 300 ng/ml for chemotaxis and up to 1 μg/ml for calcium transients). The concentrations of the other agonists were: 10^{-7} M PAF; 3×10^{-7} M 5-HETE; 100 ng/ml GM-CSF; 20 nM TNFα. Chemotactic response is expressed as % of activity using the migration to MCP-3 as 100%. nd, not done.

[b] Changes in calcium concentrations (above basal level; 85 ± 20) are expressed as: + (<50 nM); ++ (50–500 nM); and +++ (>500 nM).

[c] In parenthesis is indicated the number of experiments.

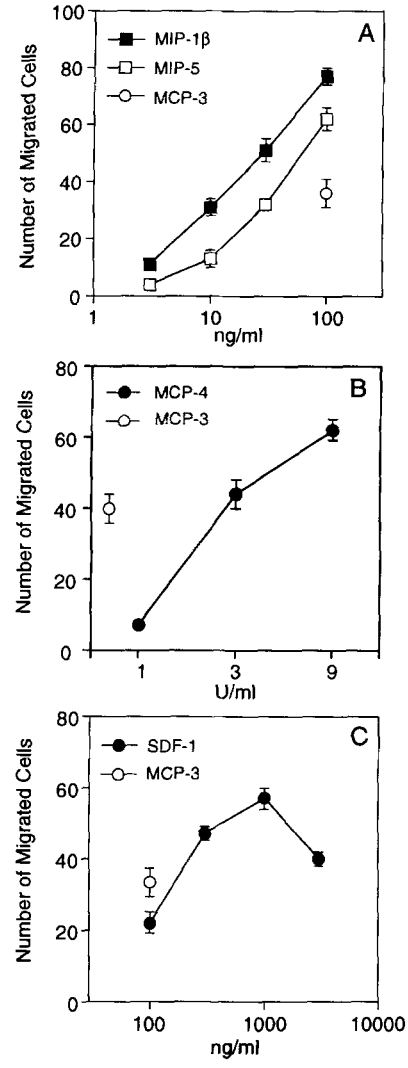

FIGURE 3. Chemotactic activity of MIP-1β, MIP-5/HCC2, MCP-4, and SDF-1 for DC. DC (1×10^6/ml) were tested for their ability to migrate across a 5-μm pore-size polycarbonate filter in response to different concentrations of chemokines. At the end of the incubation (90 min), the number of cells in five high power microscope-immersion fields was evaluated. Results of one experiment (performed in triplicate and representative of at least three independent experiments) are shown. Numbers are corrected for basal migration (against medium; 24 ± 3). Chemotaxis to MCP-4 is expressed as monocyte chemotactic units (1 U = the dilution providing 50% maximal chemotactic response of human monocytes). MCP-3 was used as a reference chemokine at the concentration of 100 ng/ml (6 U).

In this study as well as in a previous study conducted by this group, we found that MCP-3, unlike MCP-1, is a potent chemoattractant for DC. MCP-3 interacts with both CCR1 and CCR2, while MCP-1 binds only CCR2 with high affinity. We previously reported that MCP-3 acts mainly, if not exclusively, on monocytes with CCR2, a receptor shared with MCP-1 (40). Therefore, it was important to better characterize in DC the receptors for MCP-3. Figure 4 shows that in DC, displacement of labeled MCP-3 with cold MCP-3 resulted in a sigmoidal competition curve with an IC_{50} of 2.7 ± 0.6 nM (n = 4). [^{125}I]MCP-3 competition by MIP-1α and RANTES also resulted in a sigmoidal curve of inhibition with IC_{50} values of 29 ± 10 nM and 220 ± 80 nM (n = 3), respectively. Cold IL-8 (C-X-C) and MCP-1 (C-C) did not compete with the binding of [^{125}I]MCP-3 to DC (Fig. 4). These results are consistent with the use of CCR1 by MCP-3 in DC.

Induction of calcium transients in DC

Chemotactic agonists induce a rapid, PTox-sensitive, and transient rise of $[Ca^{2+}]_i$ in target cells (18, 48). In a previous study, we have shown that chemoattractants also stimulate calcium fluxes in DC (12) and that this response was completely blocked by PTox (data not shown). Figure 5*A* shows that MIP-1β, at chemotactic concentrations, increased $[Ca^{2+}]_i$ in DC. MIP-1β induced a partial heterologous desensitization to a subsequent challenge with MIP-1α (Fig. 5*A*). Conversely, RANTES cross-desensitized for MIP-1β (Fig. 5*B*). These results are consistent with the interaction of MIP-1β with CCR5 (30, 49). MIP-5/HCC2 was also active in increasing $[Ca^{2+}]_i$ in DC and cross-desensitized for a subsequent stimulation with MCP-3 (Fig. 5*D*) and MIP-1β (Fig. 5*F*). On the contrary, MCP-3 did not affect a subsequent stimulation with MIP-5/HCC2 (Fig. 5*E*), and MIP-1β only partially reduced MIP-5HCC2 response (Fig. 5*F*). These results, although indirect, suggest that MIP-5/HCC2 activates DC through the interaction with two receptors: CCR1, as previously described[4] (50), and CCR5. Three other agonists active in terms of chemotaxis, SDF-1, MCP-4, and PAF, induced a rise in $[Ca^{2+}]_i$ (Fig. 5*C*). None of the inactive chemotactic agents induced a significant and reproducible increase of $[Ca^{2+}]_i$ (Table I).

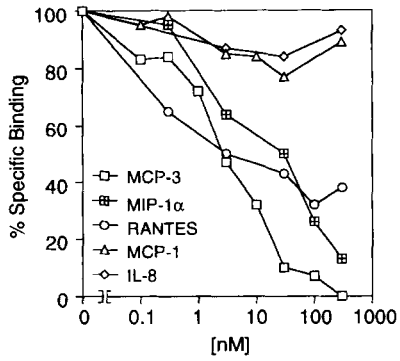

FIGURE 4. Displacement of [^{125}I]MCP-3 binding to DC by unlabeled MCP-3, MIP-1α, RANTES, MCP-1, and IL-8. Duplicate samples of 1 × 10^6/ml DC in 200 μl of binding medium were incubated with 0.5 nM [^{125}I]MCP-3 in the presence of increasing quantities of unlabeled cytokines. After incubation at 4°C for 2 h, the cells were centrifuged through a silicon oil cushion, and the radioactivity in the pellets and in the supernatants was measured. The average values of three independent experiments are shown.

Effect of C-C chemokines on endocytic activity and MLR

It has been shown that chemokines can play a role in the differentiation of hemopoietic precursors and in the activation of NK cells (51–53). Monocyte-derived DC cultured with GM-CSF and IL-4/IL-13 show high endocytic activity, measured as uptake of FITC-dextran, and potent ability to stimulate proliferation of allogenic T cells (37, 54). Therefore, it was of interest to investigate whether active chemokines could modulate, in DC, biologic functions other than chemotaxis. Figure 6*A* shows that a 30-min preincubation of differentiated DC with an optimal concentration (100 ng/ml) of MIP-1α, RANTES, MCP-3, or MCP-1 did not significantly change FITC-dextran uptake by DC. Similar negative results were observed with a longer (24 h) preincubation with chemokines (data not shown). On the contrary, in the same assay conditions, cells incubated with TNF-α for 24 h showed a decreased endocytic activity, as previously reported (54). Similarly, active C-C chemokines did not affect the ability of DC to stimulate a MLR using cord blood T lymphocytes (Fig. 6*B*).

Discussion

To initiate primary T cell-dependent immune responses, DC migrate from nonlymphoid organs into lymph nodes and spleen. The signals responsible for DC mobilization in vitro and in vivo are still largely unknown (1, 2, 8). In a previous report, we showed that MCP-3, RANTES, and MIP-1α, three C-C chemokines, were active in inducing chemotaxis and calcium fluxes in DC in vitro. On the contrary, IL-8 and IP-10, two members of the C-X-C chemokines, were inactive (12).The present study confirms and extends the previous observations to new proteins of the chemokine family and to other chemotactic agonists, some of which have only recently been described. MIP-1β, MIP-5/HCC2, MCP-4 (C-C), and SDF-1 (C-X-C) were able to induce directional migration and calcium fluxes in DC (Figs. 3 and 5), while eotaxin (C-C), lymphotactin (C), and Gro-β (C-X-C) were inactive in the range of concentrations tested (up to 1 μg/ml; Table I). PAF, a weak chemotactic factor for neutrophils and monocytes, efficiently induced chemotaxis (Table I) and calcium transients in DC (Fig. 5*C*). On the contrary, 5-HETE induced neither chemotaxis nor calcium fluxes (Table I). Thus, DC can be activated by a select number of C-C chemokines, by SDF-1, a C-X-C chemokine, and by PAF, a bioactive phospholipid. This spectrum of action overlaps, but is distinct from, that of all the other leukocyte populations (15, 18). Recently, we reported that macrophage-derived chemokine (MDC), a new C-C chemokine, preferentially activates DC (two logs more potent on DC than on mononuclear cells) (55). It is interesting to note that DC can produce chemokines (e.g., IL-8 (56), MIP-1γ (57), MDC (55), MCP-1, RANTES, and MIP-1α; S. Sozzani, unpublished observations) as well as respond to chemokines. MIP-1γ, MIP-5/HCC2, MDC, and SDF-1 are constitutively produced in different tissues[4] (46, 55, 57, 58), and it is tempting to speculate that they might play a role in DC localization in nonlymphatic tissues.

Chemokines bind to seven-transmembrane domain receptors associated with PTox-sensitive G proteins (22, 23). Consistent with this general rule, active chemokines induced a rise of $[Ca^{2+}]_i$ (Fig. 5) and were inhibited by PTox (data not shown). It was found by Northern blot analysis that DC express high levels of mRNA for CCR1, CCR2, and CCR5 receptors (Fig. 1). CCR1 is a promiscuous receptor for RANTES, MIP-1α, and MCP-3 (59, 60), three active agonists for DC (12). Accordingly, DC possess specific binding sites for [^{125}I]MCP-3, and labeled MCP-3 is displaced by cold MCP-3, MIP-1α, and RANTES (Fig. 4). CCR2 is a receptor shared by MCP-1 and MCP-3 (39, 61), and CCR5 is the receptor for MIP-1β, MIP-1α, and RANTES (30, 62). CCR3 and CCR4 were detectable only after prolonged exposure of the autoradiographs (several days vs hours for CCR1, CCR2, and CCR5). Among the C-X-C chemokine receptors investigated, CXCR1, CXCR2 (formerly IL-8A and -8B receptors) (63, 64), and CXCR4 (also known as LESTR or fusin) (29) were also found expressed in DC (Fig. 2). The C-C chemokine receptors expressed by DC fully account for the biologic activity observed with the different ligands tested with the exception of CCR2, the MCP-1 receptor, and CXCR1 and CXCR2, the IL-8 receptors. In all of the experiments performed, MCP-1 did not induce activation of DC in terms of calcium transients and chemotaxis (Table I), and these results were recently extended to DC originated from CD34$^+$ cells (S. Sozzani, unpublished observations). Recently, it was reported that CD34$^+$-derived DC possess specific binding sites for MCP-1 and migrate in response to this chemokine, although less efficiently than to MCP-3 (13). Transgenic mice that express MCP-1 in the basal layer of epidermis under the control of the K14 promoter showed local accumulation of CD45$^+$, I-A$^+$ cells that assumed a dendritic morphology in situ (65). The reason for this discrepancy is unknown. The DC used in this study showed low but detectable levels of [^{125}I]MCP-1-specific binding sites (data not shown). Similarly, DC express the mRNA for CXCR1 and CXCR2 (Fig. 2), possess specific binding sites for [^{125}I]IL-8 (4500 cpm and 1500 cpm using 0.35 nM [^{125}I]IL-8 in the absence or presence, respectively, of 100-fold excess unlabeled protein) (13), and stain with specific mAb for CXCR1 and CXCR2 (S. Sozzani, unpublished observations). However, they do not biologically respond to IL-8 and Gro-β (Table I and refs. 12, 13). Similarly, monocytes express CXCR1 and CXCR2 (Fig. 2) but do not respond to IL-8 or other CXCR2 ligands (15–18). Thus, as previously reported for a number of cell types including HL-60 and THP-1 cells (66, 67) as well as CD34-DC (13), chemokine surface receptor expression and function can exist in an uncoupled state from second messenger activation.

Active chemotactic agonists induced a transient rise of $[Ca^{2+}]_i$ (Fig. 5). Cross-deactivation of calcium transients has been used in the past to gain insights into chemokine receptors cross-utilization (22, 23). Calcium cross-deactivation studies in DC have provided results consistent with the presence of functional CCR1, CCR5 and CXCR4 (Fig. 5).

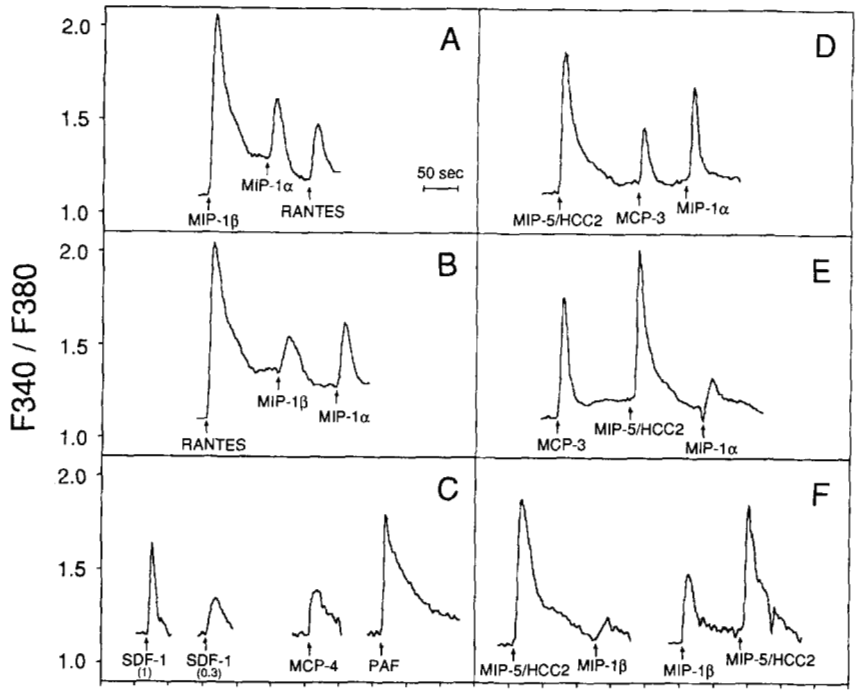

FIGURE 5. Measurement of $[Ca^{2+}]i$ in DC stimulated with chemotactic agonists. DC (10^7/ml), loaded with 1 μM fura-2 AM, were stimulated with 100 ng/ml MIP-1α, MIP-1β, RANTES, MCP-3, MIP-5/HCC2, or SDF-1 (1 and 0.3 μg/ml) or PAF (10^{-7} M). Traces from one experiment representative of at least four are shown. Results are expressed as the ratio of fluorescence at two excitation wavelengths (340 and 380 nm) and emission at 487 nm.

It has long been known that DC are highly motile in vitro and in vivo (1, 2). Attraction of immature DC precursors to peripheral tissues and migration of Ag-carrying DC to lymph nodes is essential for the induction of the immune response. Migration patterns of DC in experimental animals have been extensively studied (5–11). These studies have shown that DC migrate from nonlymphoid tissues to T cell areas of lymph organs and that LPS, GM-CSF, IL-1, and TNF are involved in their mobilization. However, the simultaneous injection of anti-TNF Ab and LPS did not prevent DC migration in vivo, suggesting that additional factors induced by LPS might be involved in DC mobility (9). Chemokines are produced by several cell types in response to all the agonists so far described as active in inducing DC mobilization in vivo (15, 18). Thus, it is possible the at least some of the chemokines found active in vitro might play a role in DC distribution in vivo. In this context, it is also interesting to note that RANTES, MIP-1α, and MIP-1β are locally produced in the lymph nodes of healthy individuals and that their levels are increased in HIV^+ subjects (68). HIV infects DC, and this cell type, strategically located at mucosal surfaces, may play an important role in the spreading of infection to $CD4^+$ T cells (69, 70). The chemokine receptors CCR5 and CXCR4 act has major fusion coreceptors for monocytotropic, duotropic, and lymphotropic HIV strains (71). These receptors are expressed in DC (Figs. 1 and 2) and very likely play a role in the infection of DC located at the virus port of entry and, subsequently, in the spreading of the virus to $CD4^+$ cells.

Migratory DC traveling between nonlymphoid and secondary lymphoid tissues are considered to undergo a maturation process, moving from a processing to a presenting stage (8). The same signals that are active in vivo (e.g., LPS, GM-CSF, TNF, and IL-1) also induce maturation of DC in vitro (54). On the contrary, chemokines failed to increase DC Ag-presenting ability and FITC-dextran endocytosis (Fig. 6). In preliminary experiments, it was also found that chemokines (MIP-1α and MCP-3) are unable to interfere with the differentiation of monocytes to DC in vitro (data not shown). Thus, at least in vitro, maturation of DC and induction

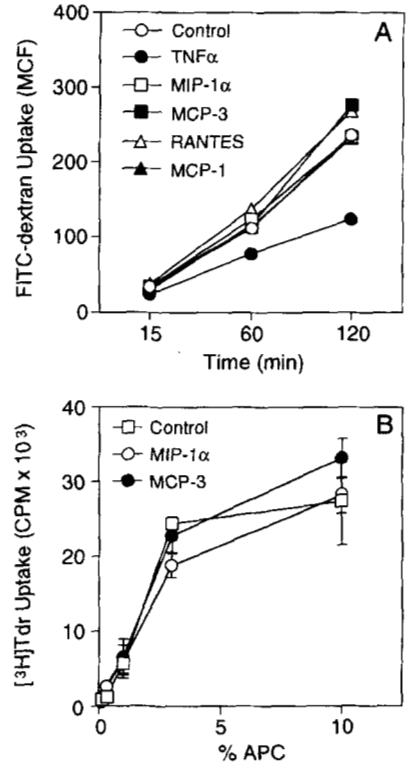

FIGURE 6. Effect of chemokines on FITC-dextran uptake and accessory function of DC. *A*, Cells were exposed to chemokines (100 ng/ml) for 30 min and then incubated with 1 mg/ml FITC-dextran for different amounts of times. In the appropriate group, cells were incubated with 20 ng/ml of TNF-α for 24 h. FITC-dextran uptake was analyzed by FACS, and the results are expressed as mean channel fluorescence (MCF). One experiment representative of two is shown. *B*, DC were incubated with 100 ng/ml of MIP-1α or MCP-3 for 1 h. Responder cells were cord blood T lymphocyte-depleted of monocytes and B lymphocytes. [^{3}H]Tdr was added during the last 18 h of a 5-day experiment. One experiment representative of two is shown.

of chemotaxis appear to be two dissociated processes caused by different classes of activators.

In conclusion, DC generated from peripheral blood precursors express receptors for and respond to a unique set of CC and CXC chemokines and lipid chemoattractants. It is likely that these chemoattractants play a crucial role in the accumulation in tissues and in the complex trafficking pattern of DC in vivo. Because of their essential role in the induction of immune responses, DC are now considered promising tools and targets for immunotherapy (3, 4). The results reported here identify molecular tools useful to direct DC traffic that can be exploited in the context of immunization strategies.

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