RANTES-Induced Chemokine Cascade in Dendritic Cells

Falko R. Fischer, Yi Luo, Moli Luo, Laura Santambrogio and Martin E. Dorf

J Immunol 2001; 167:1637-1643; doi: 10.4049/jimmunol.167.3.1637
http://www.jimmunol.org/content/167/3/1637

References
This article cites 36 articles, 15 of which you can access for free at:
http://www.jimmunol.org/content/167/3/1637.full#ref-list-1

Subscription
Information about subscribing to The Journal of Immunology is online at:
http://jimmunol.org/subscription

Permissions
Submit copyright permission requests at:
http://www.aai.org/About/Publications/JI/copyright.html

Email Alerts
Receive free email-alerts when new articles cite this article. Sign up at:
http://jimmunol.org/alerts
RANTES-Induced Chemokine Cascade in Dendritic Cells

Falko R. Fischer,*† Yi Luo, Moli Luo, Laura Santambrogio,‡ and Martin E. Dorf†‡

Dendritic cells (DC) are the most potent APCs and the principal activators of naive T cells. We now report that chemokines can serve as activating agents for immature DC. Murine bone marrow-derived DC respond to the CC chemokine RANTES (10–100 ng/ml) by production of proinflammatory mediators. RANTES induces rapid expression of transcripts for the CXC chemokines KC and macrophage inflammatory protein (MIP)-2, the CC chemokines MIP-1β and MIP-1α, and the cytokines TNF-α and IL-6. Synthesis of KC, IL-6, and TNF-α proteins were also demonstrated. After 4 h, autoinduction of RANTES transcripts was observed. These responses are chemokine specific. Although DC demonstrated weak responses to eotaxin, DC failed to respond to other chemokines including KC, MIP-2, stromal-derived factor-1α, MIP-1β, MIP-1α, monocyte chemoattractant protein-1, T cell activation gene 3, or thymus-derived chemotactic agent 4. In addition, RANTES treatment up-regulated expression of an orphan chemokine receptor termed Eo1. Chemokine induction was also observed after treatment of splenic DC and neonatal microglia with RANTES, but not after treatment of thymocytes or splenocytes depleted of adherent cells. TNF-α-treated DC lose responsiveness to RANTES. DC from mice deficient for CCR1, CCR3, and CCR5 respond to RANTES, indicating that none of these receptors are exclusively used to initiate the chemokine cascade. RANTES-mediated chemokine amplification in DC may prolong inflammatory responses and shape the microenvironment, potentially enhancing acquired and innate immune responses. The Journal of Immunology, 2001, 167: 1637–1643.

Chemokines are small chemoattractant cytokines that induce leukocyte accumulation at inflammatory sites or regulate leukocyte trafficking through lymphoid tissues. Based on the spacing of two cysteines in the N-terminal regions of the molecules, chemokines are grouped into different families, most notably the CC and CXC chemokines. RANTES, also termed CCL5, is a proinflammatory CC-chemokine that has an important role in multiple chronic inflammatory conditions. RANTES is the most potent natural inhibitor of M-tropic HIV-1 infection (1). Chemokines deliver signals through seven-transmembrane-spanning receptors (2–4). RANTES displays high affinity binding and signaling through multiple independent chemokine receptors including CCR1, CCR3, and CCR5 (5).

Dendritic cells (DC) are the most potent APCs and the principal activators of naive T cells. DC form a network of phenotypically and functionally distinct populations that initiate and differentially regulate immune responses in primary and secondary immune organs (6–8). The proper function of immune surveillance requires well-coordinated mechanisms to guide lymphocytes and APCs through peripheral tissues and into secondary lymphoid organs. After capture of Ag in the periphery, DC mature and modulate their chemokine receptor profile, down-regulating CCR1 and CCR5, which recognize RANTES and other proinflammatory chemokines, and up-regulating CCR7, which directs cells into the secondary lymphoid tissues (9). After stimulation DC migrate to the secondary lymphoid organs to initiate immune responses (9). Following exposure to proinflammatory cytokines or bacterial components like LPS, DC produce high amounts of different chemokines in a time-ordered fashion dependent on the nature of the stimulus (10, 11). These and other studies exploring selective chemokine production by DC (12, 13) suggest that temporally and spatially focused production of chemokines contribute to DC function during immune responses (11). We have now tested a panel of CC and CXC chemokines for their ability to induce chemokines in murine bone marrow-derived DC. DC selectively respond following RANTES stimulation by inducing an amplification cascade that results in the synthesis of several proinflammatory chemokines and cytokines.

Materials and Methods

Mice

Female SJL/J or BALB/c mice were purchased from The Jackson Laboratory (Bar Harbor, ME). CCR1- and CCR3-deficient mice were bred on a mixed 129/BALB background (14, 15), whereas CCR2- and CCR5-deficient mice were on a mixed 129/SvJBL background (16). Mice were maintained in accordance with guidelines of the Committee on Animals of the Harvard Medical School and those prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Resources, National Research Council (Department of Health and Human Services Publication, NIH 85-23, 1985).

Reagents

Recombinant murine TNF-α, IL-1β, RANTES, KC, macrophage inflammatory protein (MIP)-2, MIP-1α, MIP-1β, eotaxin, and stromal-derived factor-1α were purchased from R&D Systems (Minneapolis, MN). Monocyte chemoattractant protein (MCP)-1 and TCA3 were purchased from BD Pharmingen (San Diego, CA) and GM-CSF from RDI Research Diagnostics (Flanders, NJ). Recombinant mouse thymus-derived chemotactic agent 4 (TCA4) was prepared as detailed elsewhere (17). LPS was obtained from Sigma (St. Louis, MO). All chemokines were passed over Detoxi-Gel (Pierce, Rockford, IL) to reduce potential endotoxin contamination.
Preparation of bone marrow-derived DC

DC were prepared by the method of Lutz et al. (18) with some modifications. Femurs and tibiae of 6- to 12 wk-old SJL/J or BALB/c mice were aseptically removed and cleared from surrounding muscle. Chemokine-deficient mice were usually 8- to 20-wk old. After the bones were cut and placed in cold medium the marrow was flushed out with a syringe. The medium used for all cultures, designated complete medium (CM), was DMEM (Life Technologies, Grand Island, NY) supplemented with 5% heat-inactivated FBS, 2 mM l-glutamine, 1 mM sodium pyruvate, 10 mM HEPES buffer, and 1× nonessential amino acids (Sigma). Bone marrow was seeded at 5 × 10^6 leukocytes (no selection and no lysis of red cells) in a 100-mm petri dish (catalog no. 25384-208; VWR, West Chester, PA) in 8 ml CM with 15 ng/ml recombinant mouse GM-CSF for 3 days. Cells were fed with 8 ml CM containing 15 ng/ml GM-CSF on day 6. On day 8, 8 ml of medium was collected, the cell pellet was resuspended in 8 ml of the above medium, and the cells were returned to culture. Cells were also fed on days 10 and 12 as above, but the GM-CSF dose was reduced to 5 ng/ml. Alternatively, after red cell lysis with lysis buffer (Sigma) bone marrow cells were plated at a density of 0.75–1 × 10^7 cells/well in 24-well plates in CM with 5 ng/ml GM-CSF and fed every other day. On day 10 the dose of GM-CSF was reduced to 2.5 ng/ml. Nonadherent DC were usually collected on day 12 for chemokine induction assays. From both procedures the nonadherent cells at that time were a homogenous population of large cells with long dendrites, which stained positively with Abs for CD11b, CD11c, DEC205 (similar to reported staining data; Ref. 18), and for the empty form of class II with KL304, an Ab specific for immature DC (19). The preparations of bone marrow-derived DC were not stained with anti-CD8. Contamination with granulocytes was <5% as detected by staining with anti-Ly-6G (BD Pharmingen). All chemokine induction assays were performed in CM without serum, generally 0.8–1 × 10^7 DC were plated in a 12-well plate in 500 μl of medium (800 μl for overnight) plus the indicated concentrations of recombinant chemokine, cytokine, or LPS. Incubations were for 6 h or as indicated.

Preparation of splenic DC

Splenic DC were isolated from C57BL/6 mice ~12 days after s.c. injection of 1 × 10^6 Flt3 ligand-producing B16 tumor cells (20). Flt3 ligand-producing tumor stimulates a 100-fold increase in DC recovery from the spleen. Spleens were treated with 1 mg/ml collagenase (Sigma) mechanically disrupted using the rough ends of two microscope slides, and plated in tissue cultures dishes in CM. After a 2-h adherence, nonadherent cells were discarded and the remaining cells were cultured in 5 ng/ml GM-CSF in CM. After an additional 16 h culture nonadherent cells were highly enriched DC. These cells have an “immature” DC phenotype staining positively with Abs for CD11b, CD11c, DEC205, and for the empty form of class II with KL304. The splenic DC population was homogeneous for CD8, ~50% of cells stained with anti-CD8 Ab.

Preparation of microglia

Mixed glial cell cultures were prepared as detailed previously (21). Microglia were harvested by shaking the cultures between days 20 and 26. The population stained positively for CD11b and CD45.

Preparation of thymocytes and splenocytes

Thymus and spleens of 6- to 8-wk-old SJL/J mice were removed and mechanically disrupted as detailed above but without collagenase treatment. Splenocytes and thymocytes were cleared from most macrophages by plastic adherence for 2 h and stimulated with chemokine or with Con A (2.5 μg/ml) for 6 h.

Preparation of RNA

RNA was extracted with TRIzol reagent (Life Technologies) according to the manufacturer’s protocol. The concentration of RNA was determined by spectrophotometry at 260 nm. RNA samples were stored at −80°C.

Rnase protection assay (RPA)

RPA was performed using the RiboQuant multiprobe RPA system (PharMingen, San Diego, CA) and the manufacturer’s protocol. The chemokine template DNA sets consisted of lymphotactin, RANTES, eotaxin, MIP-1α, MIP-1β, MIP-2, IP-10, MCP-1, TCA3, and two housekeeping genes: large ribosomal subunit protein 32-3A (L32) and GAPDH. A customized set of chemokine receptor templates detected housekeeping genes plus CCR1, CCR5, CXCR chemokine receptor (CXCX) 4, CXC3CRI, and the orphan receptor Eo1 (GenBank accession no. AF030185, AF316576). Briefly, a 32P(UITP)-labeled antisense RNA probe was prepared using T7 RNA polymerase. Target RNA (1.5–2.0 μg) was hybridized overnight followed by digestion of unprotected RNA with RNase. The treated RNA was extracted, and the samples were loaded onto an acrylamide/urea sequencing gel next to labeled probes that served as size markers. Gels were digitally scanned using a phosphorimager, and single bands were normalized based on densitometric values to one of the housekeeping genes using ImageQuant software with the “local average” background correction (Molecular Dynamics, Sunnyvale, CA).

RT-PCR

Single-stranded cdNA was synthesized from RNA using the Superscript Preamplification System for First Strand cDNA Synthesis (Life Technologies, Gaithersburg, MD) with the following modifications: 2 μg total RNA was treated with 15 U bovine pancreas DNase-1 (Sigma) for 20 min at 25°C in an 18 μl volume containing 1× PCR buffer and 2 mM MgCl2. Enzyme was inactivated by incubation with 2 μl 25 mM EDTA at 65°C for 10 min. Random hexamers (3 μl) were added and annealed to the RNA at 70°C for 10 min. The reverse transcription reaction was performed according to the manufacturer’s protocol for a single reaction. One-half the reaction mix was mixed to a different tube to serve as a no-reverse transcriptase control. The other aliquot was incubated with 100 U of SuperScript II reverse transcriptase. PCR was performed in a 20 μl reaction mixture with 0.5 μl cdNA, 0.5 μM of each primer, and the manufacturer’s Taq DNA polymerase conditions (Quagen, Valencia, CA). The KC specific primers were GGAAGTACCATGATCCACGACCCGG and GCTCTAGTGTTACTGGGGACACCTTTCAG; the CCR7 specific primers were CTCTGAGGAGCGGTCTGATG and CTTCTGGAGGCGGCCTGTAG; and the β-glucuronidase primers were ATCCGAGGAAAGGTCCTAGG and GAGCAGAGAAAGGTCTAGTGG. The PCR program included preincubation at 94°C for 2 min, amplification with 30 cycles at 94°C for 45 s, annealing at 55°C for 45 s, extension at 72° for 45 s, and a final 72°C extension for 5 min. PCR products (6.5 μl) were visualized on 3% agarose minigels. cdNA from peritoneal exudate cells served as a positive control.

ELISA

DC (7 × 10^6) were cultured in 120 μl serum-free CM with the indicated amounts of chemokine, cytokine, or LPS in flat-bottom 96-well plates for 48 h. The anti-KC and anti-IL6 capture and biotinylated detection Abs were obtained from R&D Systems. Abs for quantitation of TNF-α were purchased from BD Pharmingen. The ELISAs were performed in accordance with manufacturer’s directions or as detailed previously (21, 22).

Results

Proinflammatory cytokines and bacterial products are known to induce chemokine synthesis in DC. However, very little is known about how chemokines regulate production of other proinflammatory mediators in DC. We addressed this point by incubating mouse bone marrow-derived DC with different CXC and CC chemokines. Production of the mouse CXC chemokine, KC, was used as a read-out because it is a sensitive indicator of chemokine protein synthesis following stimulation with proinflammatory mediators (21, 23). LPS, TNF-α, and IL-1β were used as positive controls inducing 29–146 ng/ml KC (Fig. 1A). RANTES was the only chemokine capable of inducing significant levels of KC protein synthesis (41–84 ng/ml). The other CC chemokines (TCA3, TCA4, MIP-1α, MIP-1β, and MCP-1) and CXC (MIP-2, stromal-derived factor-1α) tested failed to induce KC. DC treated with GM-CSF, a growth factor that induces DC differentiation of bone marrow precursors, failed to induce KC synthesis. All reagents were pretreated with Detoxi-Gel to remove any contaminating endotoxin. In addition, a low dose of LPS (10 pg/ml) was included, as this is the highest level of contamination reported by the suppliers of the recombinant chemokine proteins. LPS (10 pg/ml) also failed to stimulate KC production, thereby excluding endotoxin contamination as responsible for these observations. The kinetics of KC induction by RANTES was investigated on the transcriptional level (Fig. 1B). Stimulation of DC with 100 ng/ml RANTES for 60 min was sufficient to induce KC transcripts as detected by RT-PCR. Optimal levels of PCR product were detected after 1.5–3 h.

Downloaded from http://www.jimmunol.org/ by guest on April 18, 2017
As a second step we investigated whether RANTES was capable of inducing chemokines other than KC. MIP-2, MIP-1β, and MIP-1α RNA were rapidly induced after RANTES stimulation for 1.5–3 h. In autocrine fashion, RANTES message was also up-regulated after 3–6 h (Fig. 2A). RANTES autoinduction was sustained and more pronounced after longer incubation periods (24 h) when induction of MIP-2, MIP-1β, and MIP-1α had decreased (data not shown). This amplification of chemokine transcripts was mediated by a heat-labile ligand as boiled RANTES (100 ng/ml) failed to stimulate chemokine production (Fig. 2A). To determine the minimal dose required for chemokine induction DC were incubated with 1, 10, or 100 ng/ml RANTES (Fig. 2B). RPA analysis showed that MIP-2 transcripts were readily detected after stimulation with 10 ng/ml of RANTES, whereas detection of MIP-1β or MIP-1α transcripts required higher ligand concentrations (100 ng/ml) (Fig. 2B).

The specificity of DC chemokine induction was also examined at the transcriptional level. RPA was performed with RNA samples from DC stimulated with 100 ng/ml RANTES, eotaxin, MIP-1α, MIP-1β, or TCA4. RANTES treatment increased chemokine RNA levels for MIP-2, MIP-1α, and MIP-1β by 3.3- to 4.7-fold (Fig. 2C). Eotaxin also enhanced transcription (1.7- to 2.6-fold), but chemokine induction was consistently less potent than with RANTES. Incubation with MIP-1α, MIP-1β, or TCA4 failed to up-regulate any of the chemokines assayed (Fig. 2C). In separate experiments, treatments with KC, MIP-2, and MCP-1 also failed to increase the levels of chemokine transcripts (data not shown).

Having shown that RANTES was capable of inducing chemokine expression in DC we next examined whether RANTES also stimulated cytokine expression. Rapid (3 h) up-regulation of IL-6 and TNF-α transcripts was noted (Fig. 3A). Boiled RANTES did not stimulate cytokine synthesis over control levels. The expression of TGF-β1 did not significantly change after chemokine treatment indicating the selectivity of gene regulation. RANTES treatment also stimulated production of IL-6 and TNF-α proteins (Fig. 3, B and C). Incubation of DC with eotaxin stimulated synthesis of small amounts of IL-6 (150 pg/ml), but synthesis of TNF-α protein was not detected (Fig. 3, B and C). Stimulation with TCA4 failed to activate synthesis of either cytokine.

Changes in chemokine receptor expression during DC maturation promote the trafficking of DC from peripheral tissues to secondary lymphatic organs. We investigated whether RANTES was...
able to modulate chemokine receptor expression. Treatment of DC with 100 ng/ml RANTES for 4 h up-regulated transcription of Eo1 (Fig. 4). Eo1 (GenBank accession no. AF030185 and AF316576) is a polymorphic orphan chemokine receptor with 44–46% amino acid homology to the RANTES receptors CCR1, CCR3, and CCR5. Eo1 message was described as an inducible marker of an LPS-stimulated macrophage cell line (24). Normalized RPA data revealed a 7-fold up-regulation of Eo1 in DC following treatment with TNF-α (n = 3, p < 0.005), a 5-fold increase with RANTES (n = 4, p < 0.02), but no significant change in Eo1 expression following KC treatment. RNA levels for CCR1 and CCR5 did not change significantly after a 4-h TNF-α treatment as previously reported (11), but were reduced after 24 h (data not shown). There was a tendency for down-regulation of CXCR4 following RANTES and TNF-α treatment, but the values did not reach statistical significance.

We next investigated whether RANTES-induced chemokine production could be observed in other cell types. Splenic DC were obtained from mice injected with a Flt3 ligand-producing melanoma. DC generated by this procedure express empty MHC class II molecules, a characteristic of immature DC (19). RNA from these splenic cells also exhibit very high background RANTES expression; however, after incubation with 100 ng/ml RANTES for 6 h MIP-2, MIP-1β, and MIP-1α transcripts were up-regulated by 3- to 6-fold (Fig. 5). We also tested neonatal microglia because

FIGURE 3. RANTES induces cytokines. A, DC were treated with RANTES (100 ng/ml for 3 h) and then harvested for RNA preparation. Samples were tested by RPA. One group was treated with boiled RANTES as a control. Relative intensities of protected bands normalized vs the housekeeping gene GAPDH are presented. B, Comparisons of the ability of 100 ng/ml RANTES (○), eotaxin (●), and TCA4 (▲) to release TNF-α protein in 48-h conditioned medium by ELISA. Data represent averages from two independent preparations of DC. C, Comparisons of the ability of 100 ng/ml RANTES (○), eotaxin (●), and TCA4 (▲) to release IL-6 protein in 48-h conditioned medium by ELISA. Data represent averages from 2–3 independent preparations of DC.

FIGURE 4. RANTES up-regulates the orphan receptor Eo1. Bone marrow-derived DC were stimulated with 100 ng/ml of the indicated stimulus for 4 h, then RNA was analyzed by RPA. A customized chemokine receptor template set was used (CCR1, CX3CR1, CXCR4, CCR5, and Eo1). One representative experiment of four is presented.

FIGURE 5. Tissue specificity. RNA samples from RANTES (100 ng/ml for 6 h)-treated splenic DC derived from C57BL/6 mice injected with a Flt3 ligand-producing B16 tumor line, neonatal SJL/J microglia cells, and SJL/J thymocytes were compared by RPA. One group of thymocytes was treated for 6 h with 2.5 μg/ml Con A.
these APCs of the CNS exhibit some morphological and functional similarities with DC (25–27). Bone marrow-derived DC and microglia responded similarly following RANTES treatment, displaying 2- to 19-fold increases in chemokine transcripts (Fig. 5). In contrast, nonadherent thymocytes and splenocytes failed to respond to 100 ng/ml RANTES as measured by chemokine amplification. The same cells responded to treatment with Con A stimulation by induction of transcripts for lymphotactin, MIP-1β, and MIP-1α (Fig. 5 and data not shown), indicating that lymphocytes coordinate chemokine expression to other stimuli but not to these concentrations of RANTES.

Because the phenotype of the bone marrow-derived DC used for the above experiments was immature (GM-CSF derived) we next addressed the question whether TNF-α-exposed, mature DC also respond to RANTES. Bone marrow-derived DC were planted onto new petri dishes with the addition of 50 ng/ml TNF-α for 36–48 h. The data indicate mature DC lost RANTES responsiveness (Fig. 6). In contrast, responsiveness to TNF-α as measured by chemokine induction was still present following cytokine-induced DC maturation. It should be noted that under the above conditions TNF-α-matured DC down-regulated CCR1 and CCR5 RNA levels but message for CCR7 was up-regulated (data not shown).

To date, three distinct chemokine receptors have been clearly associated with RANTES binding: CCR1, CCR3, and CCR5 (28). Transcripts for all three receptors are expressed on mouse DC (Ref. 29 and Fig. 4). To determine whether one of these receptors was used exclusively for RANTES-induced chemokine amplification, DC from CCR1-, CCR3-, and CCR5-deficient mice were stimulated with medium or 100 ng/ml RANTES, TNF-α (positive control), or KC (negative control). RANTES stimulated chemokine transcripts in all chemokine receptor-deficient DC populations (Fig. 7A). Similarly, RANTES induced KC protein production in DC from CCR1-, CCR2-, CCR3-, and CCR5-deficient mice (Fig. 7, B and C). The combined data indicate that RANTES does not use a single chemokine receptor (CCR1, CCR3, or CCR5) to signal. But DC may signal through multiple independent receptors or may use an alternative receptor for induction of chemokine transcripts.

Discussion
DC are the sentinels of the immune system. DC located in peripheral tissues play a central role in immune surveillance. The majority of DC from the periphery share common characteristics, in particular the capacity to initiate innate immune responses (11). Functional DC activity requires the combination of phagocytic activity and the presence of non-Ag-specific recognition molecules, such as Toll-like receptors, complement receptors, or chemokine receptors. The synergism among all these molecules allows DC to phagocytize foreign Ags, recognize danger signals, and migrate to lymph nodes where they activate naive T cells. The full maturation of DC to competent APC is achieved during such migration. Chemokines and chemokine receptors play an important role in DC maturation (7, 11).

As part of their role in innate immunity DC are very efficient producers of cytokines and chemokines. LPS, TNF-α, and IL-1 are the classical stimuli for the induction of chemokines in DC. Until now chemokines were not considered to be a stimulus for further chemokine or cytokine release by DC. We now report that DC respond to RANTES by induction of chemokine and cytokine synthesis. RANTES was the only chemokine capable of stimulating...
potent activity among the panel of seven CC-chemokines and three CXC-chemokines tested. Treatment of DC with as little as 10 ng/ml RANTES induced KC, MIP-2, MIP-1β, and MIP-1α transcripts and KC, IL-6, and TNF-α proteins. RANTES also acted in an autocrine fashion by inducing RANTES transcripts. Most chemokine transcripts were induced in 1–3 h. The rapid induction of these messages suggests that RANTES acts directly rather than through the production of TNF-α or other intermediates that could initiate chemokine synthesis. Eotaxin was a weak stimulus of chemokine mRNA and triggered little or nondetectable levels of cytokine proteins.

This is the first report to demonstrate autocrine chemokine regulation in DC. The autocrine production of RANTES indicates the potential involvement of this chemokine in an amplification cascade. Evidence for autocrine regulation of chemokines has been observed in a variety of cell types including endothelial cells (30), monocytes (31), mesangial cells (23), and astrocytes (21). All of these systems describe the autocrine activity of a CXC chemokine, KC, or homologous molecules (IL-8 and growth-related oncogene-α). It should be noted that KC failed to induce chemokine synthesis in mouse DC. Very few reports implicated CC chemokines in autocrine pathways, but the activity of RANTES was not examined in the latter studies.

The ability of RANTES to induce a chemokine cascade appears to be cell type-specific. Immature DC and microglia possess this capacity, whereas thymocytes and splenic lymphocytes did not respond as monitored by chemokine production even though many T cells also express functional RANTES receptors. It has been reported that 100-fold higher RANTES concentrations (≥1 μM) stimulate proliferation, cytokine release, and tyrosine kinase signaling in human T cells (34, 35), but the physiologic significance of T cell responses to micromolar concentrations of chemokine remains unknown.

The DC receptor(s) responsible for these responses remain undefined. CCR1, CCR3, and CCR5 can each recognize the RANTES ligand, and all three receptors are expressed on DC (5). CCR2, CCR3, and CCR5 also interact with eotaxin (36). Although MIP-1α and MIP-1β are structurally homologous to RANTES and also bind CCR1 and CCR5 with high affinity (4), they failed to stimulate chemokine or cytokine synthesis. Thus, the exquisite specificity of the RANTES response by immature DC suggests the receptor-ligand interactions are unique. DC from mice genetically deficient for CCR1, CCR3, or CCR5 responded normally following RANTES stimulation. Thus, the possibility of a novel RANTES receptor remains although the combined signals of multiple redundant CCRs may also elicit these DC responses.

DC resident in the peripheral tissues express CCR1 and CCR5; upon cytokine-induced maturation these receptors are lost (9, 10). The loss of RANTES responsiveness in TNF-α-matured DC (Fig. 6) may be related to the fact that multiple RANTES receptors including CCR1 and CCR5 were down-regulated, thereby potentially reducing ligand binding.

As RANTES treatment did not induce CCR7 this chemokine may not be sufficient to drive DC maturation to completion (data not shown). Although the amounts of TNF-α produced by the RANTES amplification pathway are insufficient to drive DC maturation, they may contribute to the cytokine threshold required for DC maturation. This scenario offers a self-limiting model in which RANTES facilitates DC maturation and migration from inflammatory sites, but additional factors are needed to push DC maturation to completion.

RANTES treatment induces numerous changes in DC including modulation of receptor transcripts. Four hours following RANTES stimulation DC acquire transcripts for a novel orphan chemokine receptor termed Eo1. Experiments to define a chemokine ligand for the Eo1 receptor are underway.

The selective response of immature DC to RANTES could have a role in the induction, perpetuation, and exacerbation of inflammatory and allergic diseases. In this respect RANTES antagonists may be promising targets for the therapy of acute and chronic disease.

Acknowledgments

We thank Dr. Glenn Dranoff (Dana-Farber Cancer Institute, Boston, MA) for kindly providing the Flt3-ligand-transfected B16 tumor lines, and Dr. Craig Gerard (Children’s Hospital, Boston, MA) and Dr. William Kuziel (University of Texas, Austin, TX) for kindly providing the chemokine receptor-deficient mice.

References


