

Regulation of Dendritic Cell Recruitment into Resting and Inflamed Airway Epithelium: Use of Alternative Chemokine Receptors as a Function of Inducing Stimulus¹

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Dendritic cells (DC) were purified by flow cytometry from rat tracheal mucosa; they exhibited the phenotypic characteristics of immature DC including high endocytic activity, low CD80/86 expression, and in vitro responsiveness to a broad range of CC chemokines. Daily treatment of adult rats with the selective CCR1 and CCR5 antagonist Met-RANTES reduced baseline numbers of tracheal intraepithelial DC by 50–60%, and pretreatment of animals with Met-RANTES before inhalation of aerosol containing heat-killed bacteria abolished the rapid DC influx into the epithelium that occurred in untreated controls, implicating CCR1 and CCR5 and their ligands in recruitment of immature DC precursors into resting airway tissues and during acute bacterial-induced inflammation. Comparable levels of DC recruitment were observed during airway mucosal Sendai virus infection and after aerosol challenge of sensitized animals with the soluble recall Ag OVA. However, Met-RANTES did not affect these latter responses, indicating the use of alternative chemokine receptors/ligands for DC recruitment, or possibly attraction of different DC subsets, depending on the nature of the eliciting stimulus. *The Journal of Immunology*, 2001, 167: 228–234.

Dendritic cells (DC)⁶ play a major role in the surveillance of peripheral tissue sites for incoming Ag (1), a function that is dependent on their capacity for tightly controlled migration between different compartments. Recent studies on in vitro derived immature DC populations suggest that the capacity of these cells to initially home to sites of inflammation and to subsequently migrate to draining lymph nodes (DLN) is related to a switch in both expression of chemokine receptors and chemokine production. In particular, in vitro derived immature DC express CCR1, CCR2, CCR3, CCR5, and CCR6 receptors and respond to their ligands, whereas these receptors are progressively down-regulated during maturation with concomitant up-regulation of CCR7 (2–9), prompting the suggestion that different classes of chemo-

kines may control recruitment of DC into inflammatory sites vs subsequent migration to DLN. These suggestions have yet to be systematically tested in in vivo systems.

The present experiments focus on DC in the mucosa of the conducting airways, which is under continuous exposure to a wide range of pathogenic and nonpathogenic Ags present in ambient air. The lining airway epithelium of adult experimental animals and humans contains a network of DC which comprise in the range of 400–800 DC/mm² epithelial surface, comparable to the Langerhans cell network of the epidermis (10, 11). The airway epithelial DC turn over more rapidly than DC at any other peripheral tissue site with the possible exception of the intestinal wall (12), and accumulating evidence suggests that this continuous and rapid population renewal is a direct result of the intensity of local antigenic stimulation (13). Moreover, we have previously reported that local challenge of the airway mucosa with bacterial, viral, or soluble protein Ags results in the immediate recruitment of large numbers of fresh DC with kinetics equivalent to neutrophils (14, 15), underscoring the potential importance of these cells in local immune defense.

Here we present data on the ex vivo responsiveness of freshly isolated tracheal epithelial DC to CC vs CXC chemokines and demonstrate the utility of the selective CCR1 and CCR5 antagonist Met-RANTES in blocking the recruitment of DC precursors into resting airway epithelium and during acute inflammation induced by a bacterial stimulus. However, recruitment of DC in response to challenge with live virus or a soluble recall Ag was not affected by Met-RANTES, suggesting that the mechanism(s) used for attraction of DC into airway tissues during inflammation are stimulus dependent.

Materials and Methods

Animals

Inbred PVG rats were bred free of common rat pathogens in house at the TVW Telethon Institute for Child Health Research (Perth, Australia), and

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⁶ Abbreviations used in this paper: DC, dendritic cells; DLN, draining lymph nodes; MIP, macrophage-inflammatory protein; MCP, monocyte chemoattractant protein; GRO, growth-related oncogene; PMN, polymorphonuclear neutrophil; CINC, cytokine-induced neutrophil chemoattractant.

housed as conventional animals under barrier-controlled conditions. Animals 8–16 wk old were used. All animal experimentation was approved by the Institutional Animal Ethics and Experimentation Committee, operating under guidelines set by the National Health and Medical Research Council of Australia.

Monoclonal Abs and cell-staining reagents

Methods used for immunostaining, quantification of endocytic activity via uptake of FITC-dextran, and the details of mouse mAbs to rat cell surface markers, were described previously (16). Cell samples were analyzed for surface fluorescence by flow cytometry using a FACSCalibur (BD Biosciences, Mountain View, CA).

Cell preparations

Cell isolation procedures were conducted in 0.2% BSA (PBS/BSA) or a solution of 11 mM D-glucose, 5.5 mM KCl, 137 mM NaCl, 25 mM Na₂HPO₄, and 5.5 mM NaH₂PO₄·2H₂O (GKN) supplemented with 5% FCS (GKN/FCS) or 0.2% BSA (GKN/BSA) as indicated. Collagenase digests of sliced trachea were prepared by a modification of the method described in (17). Digests were depleted of endogenous macrophages by plastic adherence for 45 min in GKN/FCS and washed in GKN/BSA, and residual contaminating B cells were removed by labeling with the OX12 mAb (anti-rat κ light chain) followed by magnetic depletion using anti-mouse IgG-coated MACS beads (Miltenyi Biotec, Bergisch Gladbach, Germany). After this procedure, contaminating macrophages were undetectable and B cells were <5%. The depleted cells were then washed and incubated with GAM-PE to label residual OX12⁺ B cells, followed by blocking with 10% normal mouse serum and incubation with OX6-FITC to label MHC class II⁺ cells. OX6⁺OX12[−] DC were then purified by dual-parameter cell sorting (Epics Elite; Coulter, Orlando, FL) as detailed previously (16) using chilled GKN plus 20% FCS as the collection medium.

Immunohistochemical analysis

Tracheas were removed and immediately fixed in cold ethanol for 30 min. The tissue was then rehydrated in PBS, embedded in 100% OCT, and

frozen in liquid nitrogen-cooled isopentane. Tangential sections 8–10 μ m thick were cut on a cryostat and immunostained, and quantification of DC conducted as detailed previously (11).

Preparation, fixation, and immunostaining of lung sections and epidermal sheets, and subsequent enumeration of DC and Langerhans cells, was as detailed previously (10, 11).

Met-RANTES treatment

Met-RANTES was produced as described before (18). Rats were inoculated i.p. with 0.5 ml PBS or an equivalent volume containing varying levels of Met-RANTES, as specified in the figure legends.

In vivo challenge systems

Bacterial model. This was based on the system detailed in Ref. 14. *Moraxella catarrhalis* was grown in Mueller-Hinton broth and suspended at $\sim 10^9$ CFU/ml. The suspension was heated at 60°C for 1 h and passed through a 26-gauge needle several times to break up bacterial clumps. Rats were exposed by aerosol to the suspension for 1 h using a Tri-R inhalation exposure apparatus (Tri-R Instruments, New York, NY).

Viral model. This was based on the system used in Ref. 15. Sendai virus was grown for 3 days in the allantoic cavity of eggs and stored in allantoic fluid at -70°C until used. Adult rats were inoculated intranasally with 10^3 hemagglutination U in 50 μ l virus containing allantoic fluid. Control animals were similarly inoculated with virus-free allantoic fluid. Infection of airway epithelium was confirmed by staining with mAb WS16 against the nucleoprotein Ag of Sendai virus (provided by Dr. A. Portner, St. Jude Children's Research Hospital, Memphis, TN). There was no influx of DC or T cells into the airway epithelium of control animals.

Soluble protein recall Ag. Animals were primed i.p. with 100 μ g OVA (Sigma, St. Louis, MO) in 0.5 ml PBS containing 10 mg aluminum hydroxide (Amphojel; Wyeth-Ayerst Laboratories, Marietta, PA). After 14 days, the animals were challenged for 30 min with an aerosol of 1% (w/v) OVA in PBS.

Table I. PCR primer sequences for chemokine studies^a

Chemokine	Primer Sequence	
MIP-1 α	S (5'–3')	CCA CTG CCC TTG CTG TTC
	AS (3'–5')	TGA TGT ATT CTT GGA CCC AGG
	Product size: 243 bp GenBank accession number: U06435	
MIP-1 β	S (5'–3')	TTC TGC GAT TCA GTG CTG TC
	AS (3'–5')	ACT CAT TGA CCC AGG GCT C
	Product size: 205 bp GenBank accession number: U06434	
MIP-2	S (5'–3')	CAA TGC CTG ACG ACC CTA C
	AS (3'–5')	CAG TTA GCC TTG CCT TTG TTC
	Product size: 194 bp GenBank accession number: S77604	
gro/CINC	S (5'–3')	ACC CAA ACC GAA GTC ATA GC
	AS (3'–5')	TGA GAC GAG AAG GAG CAT TG
	Product size: 645 bp GenBank accession number: D11444	
MCP-1	S (5'–3')	CAG GTG TCC CAA AGA AGC
	AS (3'–5')	CTG AAG TCC TTA GGG TTG ATG
	Product size: 167 bp GenBank accession number: M57441	
β -Actin	S (5'–3')	ATG CCA TCC TGC GTC TGG ACC TGG C
	AS (3'–5')	AGC ATT TGC GGT GCA CGA TGG AGG G
	Product size: 607 bp GenBank accession number: J00691	

^a S, Sense; AS, antisense.

DC chemotaxis

The assay system was as detailed in Ref. 15. Briefly, DC were enriched to $\geq 92\%$ purity from collagenase digests of respiratory tract tissue by flow cytometry as detailed in Ref. 16. Medium (RPMI plus 2.5% FCS), 600 μ l, containing putative chemoattractant was placed in the lower chamber of a Costar Transwell, and 10^5 enriched DC in 100 μ l medium were placed in the insert; after incubation for 1 h at 37°C , the top surface of the inserts was washed free of cells and the insert was fixed in cold ethanol for 10 min. The polycarbonate membranes were excised, and the contralateral surface immunostained with mAb Ox6; migrating cells were observed under a $\times 25$ objective and enumerated as mean number cells/high power field.

The chemoattractants used were: 10% zymosan-activated normal rat serum (the active agents being complement cleavage products, in particular C5a); fMLP (10^{-8} M; Sigma); rat monocyte chemotactic protein (MCP)-1 (CCL2), growth-related oncogene (GRO)/cytokine-induced neutrophil chemoattractant (CINC) (CXCL1), GRO- β (CXCL2) (all at 100 ng/ml), rat RANTES (CCL5) (200 ng/ml), and murine eotaxin (CCL11) (100 ng/ml; Peprotech (London, U.K.); human MCP-4 (CCL13) (100 ng/ml; Glaxo Wellcome, Geneva, Switzerland); and murine macrophage-inhibitory protein (MIP)-3 α (100 ng/ml; Serono, Geneva, Switzerland).

RT-PCR

Total RNA was prepared from samples of $\sim 10^5$ DC (purity, 92–94%) by RNeasy B extraction (Biogenesis, Poole, U.K.) according to the manufacturer's instructions, and mRNA was reverse transcribed to cDNA. Amplification of mRNA specific for chemokines was performed by PCR using cDNA serially diluted in reaction buffer to ensure that PCR products were being analyzed under nonsaturating conditions. Amplification of β -actin mRNA was also conducted as an internal control for efficiency of RNA extraction and reverse transcription. The sequence of PCR primer pairs used are listed in Table I below. PCR products were separated and visualized on an ethidium bromide-stained agarose gel. PCR included RT control (no RNA) and a PCR-negative control (not shown), and as a positive control cDNA from alveolar macrophages hyperstimulated with LPS/IFN- γ .

Results

Airway mucosal DC characterization

Ox6 $^+$ airway DC were sorted to $\geq 96\%$ homogeneity using gating parameters shown in Fig. 1A; Ox6 expression within the population is shown as a histogram in Fig. 1D. Endocytic activity was assessed as per Ref. 16 comparing uptake of FITC-dextran at 4°C (Fig. 1B; background binding control) vs 37°C (Fig. 1C) for 10 min in the presence of trypan blue to quench cell surface fluorescence. On the order of 50% of the DC exhibited high levels of endocytosis. Fig. 1, E and F, illustrates expression of CD86 and CD80, respectively. The hallmark features of this DC population are thus moderate to high expression levels of MHC class II and a similar distribution of endocytic activity, coupled with low levels of expression of costimulator molecules CD80 and CD86. These features are consistent with the presence within this population of a high proportion of "immature" DC.

Data illustrated in the figures and Table II are derived from groups of five animals and are shown as mean $\pm$ SEM; respective results were verified by replication ≥ 3 times except for experiments in Fig. 6 which was performed twice with comparable findings.

Recruitment of DC into resting airway epithelium

In the experiments in Fig. 2, adult control rats were dosed with Met-RANTES as shown for 1, 2, 4, or 8 days before sacrifice, and airway intraepithelial DC density was assessed in immunostained frozen tracheal sections. Continuous treatment with the chemokine receptor antagonist for 4 days resulted in a 48% reduction in airway epithelial DC density, and a further 4 days of treatment extended the reduction to 60–65%. In one series of experiments, the surface phenotypes of the airway DC that persist in the face of

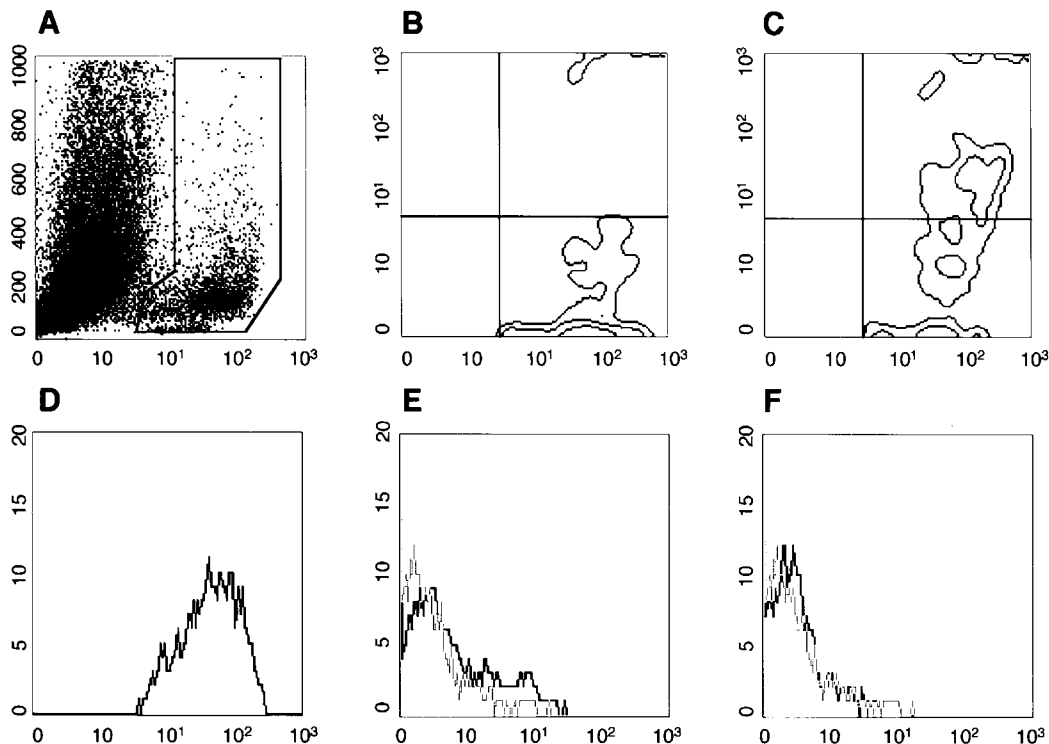


FIGURE 1. Characterization of airway mucosal DC from rat trachea. Ox6 $^+$ cells were sorted from tracheal digests to $\geq 96\%$ purity as detailed in *Materials and Methods*. A, Side scatter (x-axis) vs Ox6 staining (y-axis) showing gate setting used for DC acquisition. B, Endocytosis of FITC-dextran (x-axis) vs Ox6 (y-axis); 40°C , 10 min in the presence of trypan blue. C, Endocytosis at 37°C as per B. D, Ox6 staining. E, CD86 expression (dark line; mAb 24F) on Ox6 $^+$ DC overlaid with isotype control (light line). F, CD80 expression (mAb 3H5) on Ox6 $^+$ DC overlaid with isotype control as per E.

Table II. Failure of Met-RANTES to modulate DC recruitment in response to airway challenge with live virus or a soluble protein recall Ag

Treatment	DC/mm ² Tracheal Epithelium (days postviral infection)			
	0	3	5	8
i.n. ^a virus + daily PBS i.p.	505 ± 70	963 ± 101	1850 ± 80	1226 ± 175
i.n. virus + daily met-RANTES i.p.	521 ± 65	893 ± 65	1690 ± 66	1630 ± 37

24 h post-OVA-soluble Ag challenge	
PBS aerosol	472 ± 70
OVA aerosol	764 ± 68
OVA + Met-RANTES	789 ± 122

^a i.n., intranasal.

Met-RANTES treatment were compared with the starting population. The surface markers assessed were MHC class II (mAb OX6), CD86 (mAb 24F), ICAM-1 (mAb IA-29), and CD11b/c (OX42), as detailed in Ref. 16. No consistent differences were detected (data not shown).

Fig. 3 contrasts the effects of Met-RANTES on DC population density in resting airway (tracheal) epithelium vs peripheral lung and epidermis of the same animals. Eight days of continuous treatment with the chemokine receptor antagonist reduced baseline population levels in the airway and peripheral lung by on the order of 60 and 30%, respectively, but had no detectable effects on the Langerhans cell network in the epidermis.

Airway intraepithelial DC recruitment in response to an inhaled bacterial stimulus

Exposure of adult rats to an aerosol of heat-killed *Moraxella catarrhalis* triggers rapid recruitment of a wave of DC into the airway epithelium (Fig. 4) as reported earlier (1). Preinjection of animals with ≥ 10 μ g Met-RANTES 30 min before aerosol exposure results in dose-dependent attenuation of the DC response, as measured 24 h later. In addition, follow-up experiments (not shown) were performed involving assessment of DC numbers at the 3- and 6-h time points post *M. catarrhalis* exposure in the presence and/or absence of Met-RANTES. These indicated that the small amount of DC recruitment that occurred in the treated animals peaked within the initial 3-h period and that DC numbers remained stable over the ensuing 21 h.

Other experiments (not shown) indicated that a dosage of 50 μ g Met-RANTES reproducibly inhibited $\geq 85\%$ of the DC influx, and this was used for subsequent experiments. As shown in Fig. 5, administration of 50 μ g Met-RANTES 24 h before *M. catarrhalis* challenge was essentially as effective as administration immediately before exposure, and inhibition was still observed 3 days after a single treatment with the chemokine receptor antagonist.

Airway DC recruitment in response to challenge with live virus or a soluble recall protein

As shown in Table II, both of these challenge protocols result in significant DC recruitment into the tracheal epithelium. However, unlike the situation noted above with *M. catarrhalis*, DC recruitment in response to Sendai virus or OVA challenge was not sensitive to Met-RANTES treatment.

In vitro chemokine production and responsiveness of freshly prepared resting airway DC and airway DC 24 h after *M. catarrhalis* exposure

The experiments in Fig. 6 contrast the responsiveness of rat tracheal DC to a range of stimuli comprising complement cleavage

products (generated via zymosan activation of normal rat serum), FMLP, and a selected range of CC and CXC chemokines. Responsiveness was consistently observed to all agents tested except the CXC chemokines.

In parallel, production of cytokine-specific mRNA was assessed in highly purified tracheal DC by RT-PCR using the range of rat primer sequences available to us. Those tested were MIP-1 α , MIP-1 β , MIP-2, MCP-1, and GRO/CINC. Message production for all the latter was detected in resting DC (Fig. 7), and comparable expression of the same message species was observed in DC collected post-*M. catarrhalis* exposure.

Discussion

The controlled recruitment of DC into sites of inflammation and their subsequent migration to DLN play a central role in host defense. Our current understanding of how this process is regulated is based almost exclusively on studies focusing on responses to and production of cytokines by in vitro generated DC, sampled at different stages of differentiation/activation. These studies (reviewed in Ref. 6) collectively present a plausible model for this process based on the general principle that the migration pathways

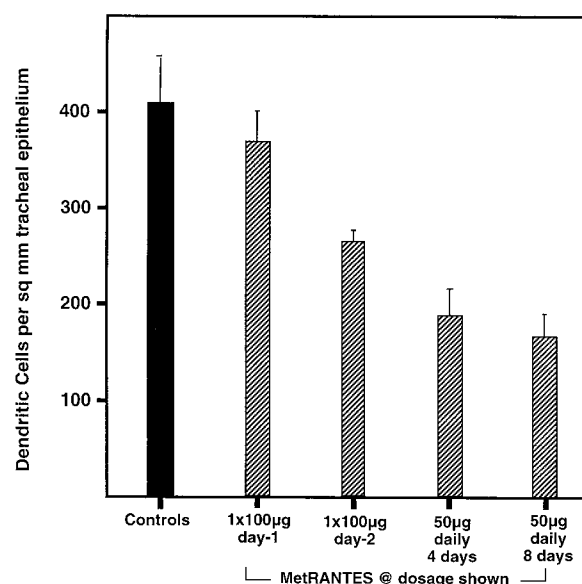
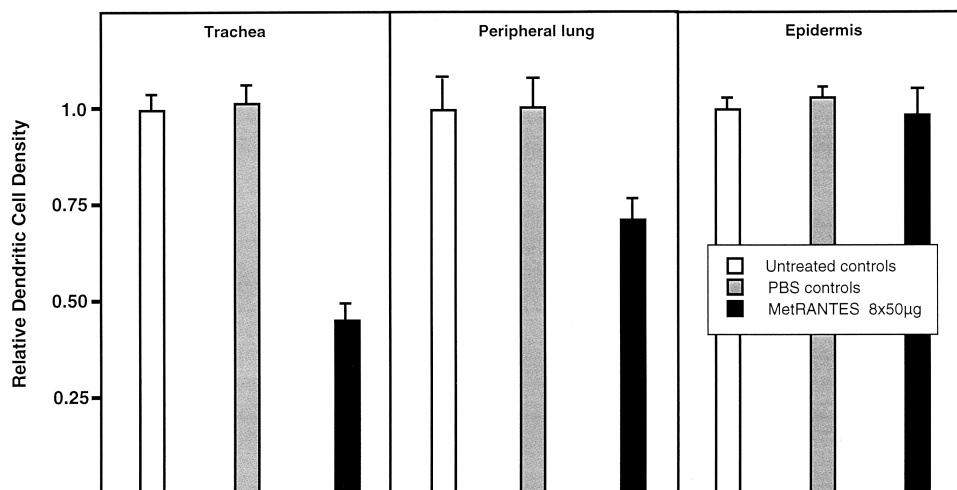


FIGURE 2. Effects of repeated Met-RANTES administration on DC numbers in the resting tracheal epithelium. Adult rats were given varying doses of Met-RANTES i.p. for up to 8 days before sacrifice, and DC density was subsequently determined in frozen sections from tracheal epithelium.

FIGURE 3. Effects of Met-RANTES on DC populations in different tissues. DC numbers were determined in lung and tracheal sections and in epidermal sheets, after 8 daily i.p. injections of PBS or PBS containing 50 μ g Met-RANTES, and compared with PBS-treated controls.



followed by DC are determined via changing patterns of cytokine receptor expression during their differentiation.

Thus, immature precursor DC are attracted into inflammatory sites via a set of chemokines for which they express receptors exclusively at this early stage of differentiation, but which are down-regulated as they mature and are replaced by others which respond to chemokine signals favoring migration to DLN, such as secondary lymphoid tissue-derived chemokine produced by lymphatic endothelial cells (19) and EBI-1 ligand chemokine produced in the T cell areas of draining nodes (3, 6, 20). However, there is very limited information available on the applicability of this model to specific tissue microenvironments in vivo or on how precursor DC are attracted into resting tissues to maintain basal levels of DC during the steady state.

This study addresses some of the relevant issues in relation to the DC within the epithelium lining the conducting airways. This DC population is of major importance to host survival because it defends the most delicate epithelial interface with the external environment, which is constantly challenged by incoming Ags. Under steady state conditions, ~85% of the airway DC population turns over at a uniquely rapid rate, being renewed every 36–48 h

by influxing precursors from the bone marrow (12). The minority (long-lived) resident population has not been studied in detail.

A series of experiments in this study examined aspects of the in vivo regulation of this normal process, using Met-RANTES, a potent CCR antagonist (18). In the human system, Met-RANTES retains high affinity binding to CCR1 and CCR5 and has weak affinity for CCR3 (21, 22). High affinity is retained for murine CCR1 and CCR5, as shown in earlier in vivo studies that demonstrated inhibition of chemotaxis of murine splenocytes by RANTES and MIP-1 β with nanomolar quantities of Met-RANTES, whereas Met-RANTES is ineffective on murine CCR3 (A. Proudfoot, manuscript in preparation). Met-RANTES is also highly effective in inhibition of chemotaxis of leukocytes in vivo in the rat (23). The principal effects of Met-RANTES in the rat are therefore most likely mediated through CCR1 and CCR5. As shown in Fig. 2, chronic administration of Met-RANTES reduces the baseline DC population at this site to ~40% of starting numbers, providing for the first time evidence that interactions between CCR1 and CCR5 and their appropriate ligands play a major role in steady state regulation of mucosal DC populations.

Further evidence in support of this suggestion was provided in the experiments of Fig. 6. Previous functional studies on DC populations from the respiratory tract have been restricted to mixed

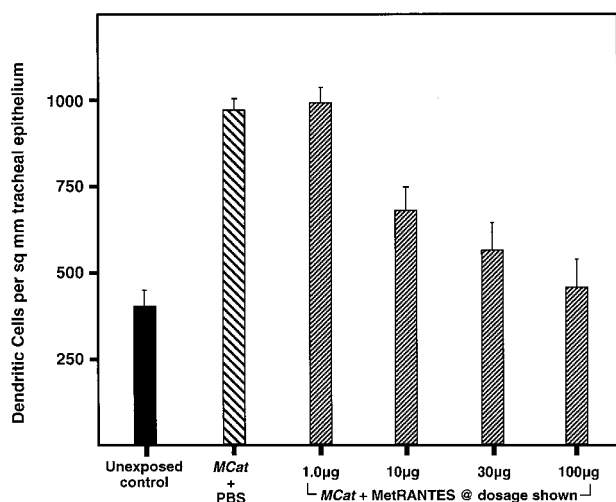


FIGURE 4. Met-RANTES-mediated inhalation of DC recruitment induced by a bacterial stimulus. DC numbers were determined in control rats and 24 h after exposure to aerosolized *M. catarrhalis* (MCat); all exposed animals received i.p. injections containing PBS or PBS plus Met-RANTES 1 h before commencement of exposure.

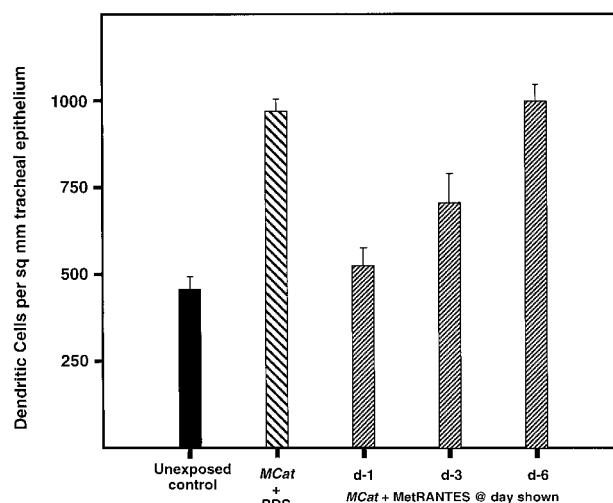


FIGURE 5. Long-lasting effects of Met-RANTES on DC recruitment. Details as per Fig. 3, except that Met-RANTES was administered once at 1, 3 or 6 days before aerosol exposure to *M. catarrhalis* (MCat).

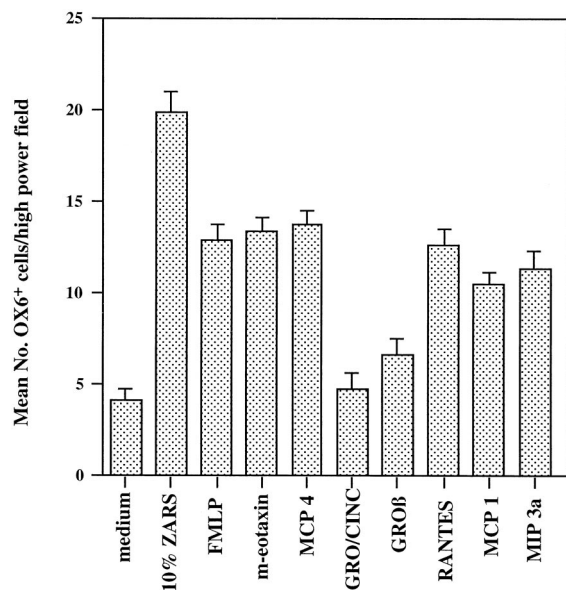


FIGURE 6. Chemotactic responses of tracheal DC. Freshly prepared tracheal DC were tested for sensitivity to a range of chemokines as detailed in *Materials and Methods*, and responses are shown as the mean number of DC adherent to the contralateral surface of replicate Costar transwells (shown as the mean number of cells per high power microscopic field $\pm$ SE) at the end of the incubation period. Migration was significantly greater than in medium controls ($p < 0.05$ – 0.01) for all chemokines tested except GRO/KC and GRO- β . 10% ZARS, Zymosan-activated normal rat serum.

populations from airways and parenchymal tissues derived by enzymatic dispersion of whole lung which by a variety of criteria express the “immature” DC phenotype (16). However, in the experiments in Fig. 6, we have for the first time prepared purified airway (tracheal) DC separately in sufficient numbers for study. These cells were responsive to the CC chemokines MCP-1 (CCR2), MCP-4 (CCR2 and 3), eotaxin (CCR3), and RANTES (CCR1, 3, and 5; see Ref. 24), suggesting a pattern of chemokine receptor expression on airway DC comparable to that of in vitro-derived DC (3–6), and also to the mixed DC population isolated from whole lung digests (16). The respiratory tract DC populations were also unresponsive to a range of CXC chemokines.

These findings are collectively consistent with those from studies that indicate CCR1 and CCR5 expression on in vitro-derived immature DC (2, 4–6). They additionally suggest that constitutive production of one or more of the respective ligands for these receptors occurs continuously within the resting airway epithelium. The nature of the cell types responsible for production of these chemokines in the steady state remains to be defined, but the presence of a range of chemokine-specific mRNA species in DC freshly isolated from trachea (Fig. 7) suggests that these cells constitute one of the potential sources (see also Ref. 5), and if so the airway epithelial DC network may to an extent be self-regulatory in the steady state.

As shown in Fig. 2, on the order of 40% of the resident DC in the resting airway epithelium are Met-RANTES insensitive. One plausible explanation for this finding is that the 40% of the population that is Met-RANTES resistant is maintained by chemokines other than those dependent on CCR1/5, e.g., CCR2, which is expressed on monocyte-derived immature DC (5, 6). This receptor binds MCP-1 which is reported to be produced constitutively by airway epithelial cells (25).

Also, the DC population in the peripheral lung and epidermis were less sensitive to Met-RANTES than their counterparts in the

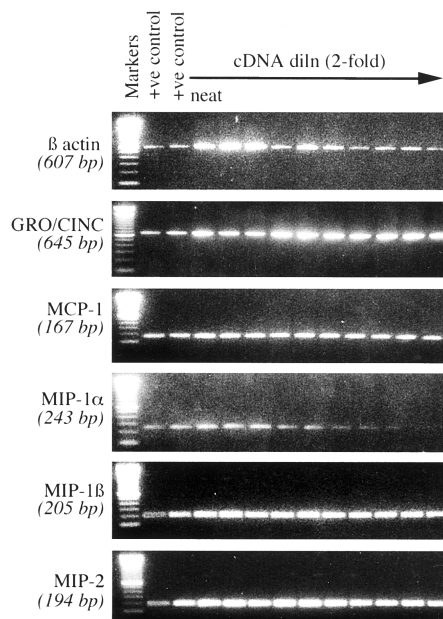


FIGURE 7. Production of chemokine-specific mRNA by tracheal DC. Total RNA was extracted from resting tracheal DC and reverse transcribed to cDNA, and amplification of chemokine-specific mRNA was performed in serially diluted samples as detailed in the text.

airway epithelium (Fig. 3). In this context, we have previously reported comparative half-life values of 1.5–2, 3–4, and >15 days, respectively, for airway, peripheral lung, and epidermal DC in the same rats (12), and the relative levels of sensitivity to Met-RANTES treatment during an 8-day period in trachea vs peripheral lung may be a direct reflection of these differences in turnover rates. Other more detailed studies on epidermal Langerhans cell turnover rates have yielded half-life figures in the range of 21–30 days (26), and the changes expected during an 8-day period in such a stable population may not be detectable with the methods used, even if the Met-RANTES were highly effective in blocking precursor recruitment.

The experiments in Figs. 4 and 5 examined airway DC recruitment during acute inflammation induced by inhalation of a bacterial stimulus. We have previously reported that the influx of DC in this system occurs with kinetics initially equivalent to polymorphonuclear neutrophils (PMN); the influx occurs as a wave of cells that peaks at ~ 2 h post-cessation of exposure and remains stable up to 48 h, before declining to baseline levels as the recruited cells migrate on to DLN (14). As shown in Fig. 4, this response is almost completely inhibitable by Met-RANTES, indicating the prime importance of CCR1/5 receptors and their ligands in this particular inflammatory response. The effects of the chemokine receptor antagonist are surprisingly long lasting, with 50% of the maximal inhibition retained when Met-RANTES was administered 3 days before Ag challenge. Although no pharmacokinetic data are available to date for modified chemokines such as Met-RANTES, it is possible that through their interactions with cell surface glycosaminoglycans they become immobilized on tissue surfaces (27), allowing their slow release into the circulation.

We have additionally reported that intranasal challenge with live Sendai virus (15, 28), or aerosol challenge of primed animals with the soluble Ag OVA (15) also recruits large numbers of DC into the airway epithelium. In the case of the bacterial stimulus, the DC were accompanied by PMN (14, 15) whereas in the viral model the accompanying cells were PMN, NK, and T cells (15, 28), vs T cells and eosinophils in the OVA model (15). In relation to the

experiments with *M. catarrhalis*, we have additionally demonstrated that Met-RANTES blocked up to 65% of the PMN response (data not shown). It is theoretically possible that PMN may be the source of the chemokines for DC recruitment and hence it is conceivable that the effects of Met-RANTES in this system are secondary to its effects on PMN recruitment. Arguing against this possibility are our previous findings indicating that initiation of DC recruitment in this model is coincident with, or even precedes, PMN recruitment (14). It is possible that T cells and eosinophils may play a role in DC recruitment process in the other models, but more detailed experiments would be required to test this proposition.

As shown in Table II, pretreatment with Met-RANTES using the protocol that virtually abrogates airway DC recruitment induced by a bacterial stimulus had no effect in the Sendai or OVA models. This indicates that CCR1/5 receptors and their ligands are not required for DC recruitment in these latter systems, suggesting therefore that alternative receptors and/or chemokines are involved, which is reflected in part by the differing cell combinations recruited by each stimulus. One set of likely possibilities with respect to DC attraction by Sendai and OVA would appear to be CCR2, CCR3, and possibly CCR6 receptors, which are expressed on in vitro-derived immature DC (2). The chemokine-binding properties of CCR5 overlap significantly with those of CCR1, whereas MCP-1 activates only CCR2 and eotaxin activates CCR3, and these two chemokines (as shown in Fig. 6 and Ref. 15) chemoattract immature lung/airway DC in rat. Note also the positive effects of MIP-3 α (which activates CCR6) in Fig. 6.

In conclusion, these studies demonstrate that attraction of DC precursors into rat airway epithelium is governed by a highly flexible chemokine receptor/ligand program. Thus, steady state DC recruitment uses CCR1/5 receptors for maintenance of up to 60% of the baseline population and presumably relies on constitutive chemokine production within the epithelium by one or more cell types which may include the resident DC themselves. During acute bacterial inflammation, reliance on CCR1/5 and their ligands for DC recruitment increases to approach 100%, but during primary viral infection or challenge with soluble recall, Ag alternative chemokine receptors/ligands are used; it is also possible that a distinct subset of DC that are not present in resting or bacterial-stimulated epithelium may be recruited in these latter situations. The ultimate result of this flexibility is that regardless of the stimulus or the nature of the final effector cell combinations that appear at the challenge site, DC appear always to comprise a significant component of the cellular influx and as such represent the ultimate "default" component of the host cellular inflammatory response. This property of DC is consistent with the suggestion that one of their prime roles is provision of the linkage between the adaptive and innate arms of the host immunoinflammatory response (29, 30).

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