Initiation and regulation of specific immune responses by keratinocytes and dendritic cells. Role of cytokines and chemokines linking innate and specific immunity
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CHAPTER 4

Differential expression of inflammatory chemokines by Th1- and Th2-cell promoting dendritic cells (DC1 and DC2). A role for different mature DC populations in attracting appropriate effector cells to peripheral sites of inflammation

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Submitted for publication
ABSTRACT

Protective immunity to pathogens depends on efficient immune responses adapted to the type of pathogen and the infected tissue. Dendritic cells (DC) play a pivotal role in directing the effector T cell response to either a protective T helper (Th) type 1 (Th1) or Th2 phenotype. Human monocyte-derived DC can be differentiated into Th1- (DC1) and Th2-promoting (DC2) DC in vitro upon activation with microbial compounds or cytokines. DC that induce both Th1 or Th2 cells are DC0. Furthermore, host defense is highly dependent on mobile leukocytes. This cell trafficking is largely mediated by interactions of chemokines with their specific G-protein coupled receptors on the surface of leukocytes. The production of chemokines by effector DC0, DC1 or DC2 remains elusive. Here we assess the differential production of both inflammatory and homeostatic chemokines by monocyte-derived DC0, DC1, or DC2. We show that DC0 and DC1, but not DC2, selectively express elevated levels of the inflammatory chemokines CCL2/MCP-1, CCL3/MIP-1α, CCL4/MIP-1β and CCL5/RANTES, as well as the homeostatic chemokine CCL19/MIP-3β. CCL21/6Ckine is preferentially expressed by DC2. Production of the Th1-attracting chemokines, CXCL9/Mig, CXCL10/IP-10 and CXCL11/I-TAC, is restricted to DC1. In contrast, the expression of Th2-associated chemokines does not strictly correlate with the DC2 phenotype, except for CCL22/MDC which is preferentially expressed by DC2. Since all the inflammatory chemokines tested are expressed constitutively by mature DC, we propose a novel role for mature DC present in inflamed peripheral tissues in orchestrating the immune response by recruiting appropriate leukocyte populations to the site of pathogen entry.

INTRODUCTION

Chemokines are small secreted proteins (6-14 kDa) that regulate leukocyte trafficking.1,2 The chemoattractant signals are integrated by the interaction of chemokines with their specific 7-transmembrane G-protein-coupled receptors on the surface of leukocytes.3 Specific routes of migration are thought to be determined by combinatorial expressions of receptors and sequential encounters with different chemotactic gradients.4

Chemokines can be functionally divided into homeostatic and inflammatory chemokines.2,5 Homeostatic chemokines are constitutively produced mainly within secondary lymphoid organs in the T or B-cell areas. In the latter, CCL19/MIP-3β and CCL21/6Ckine mediate the encounters between mature dendritic cells (DC) and the rare antigen (Ag)-
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specific T and B cells. In addition, homeostatic chemokines are also produced in discrete microenvironments of non-lymphoid tissues such as the skin and mucosa. In contrast, inflammatory chemokines such as CXCL1/growth-related oncogene (GRO)α, CXCL2/macrophage inflammatory protein (MIP)-2α, CXCL8/IL-8, CCL2/monocyte chemoattractant protein (MCP)-1, CCL3/MIP-1β, and CCL5/regulation and activated normal T cell-expressed and -secreted (RANTES) are rapidly induced in peripheral tissues upon pathogenic challenge and regulate the influx of inflammatory cells. One example is neutrophil infiltration via CXCL1/GROα, CXCL2/MIP-2α, and CXCL8/IL-8 production. While most chemokines fall into either the inflammatory or homeostatic category, some may play a dual role depending on the context in which they are produced. CCL17/thymus and activation-regulated chemokine (TARC), CCL20/MIP-3α, and CCL22/monocyte-derived chemokine (MDC) are examples of chemokines that are produced both in peripheral tissues and secondary lymphoid organs.

DC are professional antigen-presenting cells (APC) that have the unique capacity to initiate specific immune responses. Capture of Ag and initiation of immunity are distinct and spatially segregated functions carried-out by DC at different stages of development. Thus, a fundamental aspect of DC function is their capacity to migrate from inflamed tissues to the T cell areas of secondary lymphoid organs, i.e. lymph nodes (LN). DC are a major source of chemokines. Immature DC (iDC) constitutively release CCL22/MDC and CCL17/TARC. At early stages of maturation, DC produce high levels of inflammatory chemokines, such as CCL2/MCP-1, CCL3/MIP-1α, CCL4/MIP-1β, CCL5/RANTES, CXCL8/IL-8, and CXCL10/IP-10, that sustain the recruitment of circulating iDC, DC precursors, and T cells to inflamed tissue. Homeostatic chemokines, including CCL18/DC-CK1, CCL19/MIP-3β, CCL17/TARC, and CCL22/MDC, are produced or upregulated later during DC maturation, providing chemotactic signals for mature DC and for T cells in secondary lymphoid organs. Mature DC-derived CCL18/DC-CK1, CCL19/ELC, CCL22/MDC and CCL17/TARC attract naive, recently activated and central-memory T cells expressing the cognate receptors CCR7 and CCR4, CXCR5+ B lymphocytes and follicular homing T helper (Th) cells are attracted by DC-derived CXCL13/B cell-attracting chemokine-1 (BCA-1).

Likewise, T lymphocyte circulation in peripheral tissues is a complex event regulated by the local release of chemotactic stimuli and by differential expression of receptors on distinct T cell subsets. Th type 1 (Th1) cells are rich in CXCR3 and CCR5 receptors, whereas T
helper type 2 (Th2) cells express CCR3, CCR4 and CCR8. Moreover, XCL1/lymphotactin (Lptn) is chemotactic for CD8\(^-\) and CD4\(^+\) T cells, but only modestly for natural killer cells. The recently identified T regulatory (Tr) cells, producing high amounts of IL-10, coexpress both Th1- and Th2-associated receptors, with high levels of CCR8 and moderate amounts of CCR4 responding to the chemokines CCL1/1-309, CCL17/TARC, and CCL22/MDC.

However, the production of chemokines by DC0, DC1 or DC2 has not yet been addressed. Therefore, we investigated whether human monocyte-derived DC0, DC1, or DC2 display differential production of inflammatory and homeostatic chemokines. We show that DC1 and DC0 preferentially express the inflammatory chemokines CCL2/MCP-1, CCL3/MIP-1\(\alpha\), CCL4/MIP-1\(\beta\) and RANTES, and homeostatic chemokine CCL19/MIP-3\(\beta\). In contrast, CCL22/MDC and CCL21/6Ckine are preferentially expressed by DC2. We propose a novel role for mature DC present in peripheral tissues in recruiting appropriate effector leukocyte populations to the site of pathogen entry since all inflammatory chemokines tested were expressed constitutively by mature DC.

MATERIAL AND METHODS

Generation of human monocyte-derived DC from peripheral blood

Monocytes were isolated from peripheral blood, as previously described. To obtain immature DC, monocytes (2.5x10\(^6\) cells/well/5 ml) were cultured for 6 days in 6-well plates (Costar, Cambridge, MA) in Iscove’s modified Dulbecco’s medium (IMDM; Life Technologies, Paisley, U.K.) containing 1% FCS (HyClone, Logan, UT), supplemented with recombinant human (rhu) GM-CSF (500 U/ml; a gift of Schering-Plough, Uden, The Netherlands) and rhuIL-4 (250 U/ml; Pharma Biotechnologie Hannover (PBH), Hannover, Germany) as described. At day 6, the cultures consisted of uniformly CD14\(^-\), HLA-DR\(^-\), and CD83\(^-\) iDC. Cell-surface phenotype was analyzed by flow cytometry, after labelling of cells with appropriate mAb as described.

Induction of DC maturation under polarizing conditions

To induce DC maturation, day 6 DC were cultured for additional 2 days in the presence of a combination of LPS (100 ng/ml; Sigma-Aldrich, St. Louis, MA), rhuIL-1\(\beta\) (10 ng/ml; PBH) and rhuTNF-\(\alpha\) (50 ng/ml; PBH) (maturation factors, MF) to induce DC0, or a combination of
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LPS+MF and IFN-γ (10³ U/ml; a gift of P.H. van der Meide, U-CyTech, Utrecht, The Netherlands) to induce DC1, or polyriboinosinic polyribocytidylic acid (poly I:C) alone (20 µg/ml; Sigma-Aldrich) to induce DC1, or a combination of MF and PGE₂ (10⁻⁶ M; Sigma-Aldrich) to induce DC2.

Induction of IL-12p70 production by mature DC with different polarizing capacities

On day 8, mature DC were washed extensively and 2x10⁴ cells/well were stimulated in 96-well flat-bottom culture plates (Costar) in IMDM containing 10% FCS in a final volume of 200 µl with CD40L-transfected J558 cells (2.5x10⁶ cells/well; a gift of Dr. P. Lane, Birmingham, UK). Supernatants were harvested and the concentrations of IL-12p70 were measured by ELISA (see next) as previously described.¹¹

Real-time PCR

Differentially matured effector DC were washed extensively and 1x10⁶ DC were stimulated (6-well plates, Costar) with irradiated (2500 Gy) L cells transfected with CD40L (a kind gift from Dr. C. van Kooten, Dept. of Nephrology, LUMC. Leiden, The Netherlands) for 24 h. The CD40L-stimulated DC were then gently harvested and lysed.

Total RNA was prepared from cells by a single step extraction method using RNA STAT-60 (TelTest, Inc) according to the manufacturer's instructions. Each RNA preparation was treated with Qiagen DNase I. DNase treatment was determined to be complete if the sample required at least 38 PCR amplification cycles to reach a threshold level of fluorescence using β-2 microglobulin for human samples as an internal amplicon reference. Agarose gel electrophoresis and ethidium bromide staining confirmed the integrity of the RNA samples following DNase treatment. Complementary DNA was prepared from the RNA sample using Multiscribe Reverse Transcription Kit (Applied Biosystems, PE, Norwalk, CT) following the manufacturer's instructions. A negative control of RNA without reverse transcriptase was mock reverse transcribed for each RNA sample. Gene expression was measured by TaqMan² quantitative PCR (Applied Biosystems) in cDNA prepared from the cells. PCR Probes and primers were designed utilizing PrimerExpress software (Applied Biosystems). The designed primer and probe sequences are shown in Table I. Each target gene probe was labelled using FAM (6-carboxyfluorescein). The internal reference probe, human β2-microglobulin (β2μ),
was labelled with a different fluorescent dye, VIC. The differential labelling of the target gene and internal reference gene thus enabled measurement in the same well. Forward and reverse primers and the probes for both the internal reference control and the target gene were added to the TaqMan® Universal PCR Master Mix (Applied Biosystems). Each reaction contained 200 nM of forward and reverse primers plus 100nM probe for β2-microglobulin and 600 nM forward and reverse primers plus 200 nM probe for the gene of interest. TaqMan® experiments were carried out on an ABI PRISM 7700 Sequence Detection System (Applied Biosystems). The thermal cycler conditions were as follows: hold for 2 min at 50°C and 10 min at 95°C, followed by two-step PCR for 40 cycles of 95°C for 15 sec followed by 60°C for 1 min. The PCR cycles needed for FAM and VIC fluorescence to cross a threshold where a statistically significant increase in change in fluorescence (Ct = Threshold Cycle) was measured using Sequence Detector software (Applied Biosystems) according to their recommendations. Relative target gene expression was determined using the formula Rel Exp = 2^(-ΔΔCT) where ΔΔCT = (Ct Target Gene – Ct Internal Reference Control in experimental sample) – (Ct Target Gene – Ct Internal Reference Control in no template control sample).

### Table 1. Primer sequences

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<th>Reverse primer</th>
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### Array-based ELISAs

In order to measure the levels of various chemokines in mature DC-derived supernatants, we used a novel technique, array-based ELISA, which allows the simultaneous analysis of 15 chemokines in 50 μl of supernatants.32 The limits of detection of each chemokine are as follows: CCL1/1-309, 0.78 pg/ml; CCL2/MCP-1, 0.78 pg/ml; CCL3/MIP-1α, 1.56 pg/ml; CCL4/MIP-1β, 0.78 pg/ml; CCL5/RANTES, 0.39 pg/ml; CCL17/TARC, 0.39 pg/ml; CCL19/MIP-3β, 0.39 pg/ml; CCL20/MIP-3α, 0.50 pg/ml; CCL22/MDC, 0.39 pg/ml;
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CXCL1/GRO-α, 0.39 pg/ml; CXCL8/IL-8, 0.39 pg/ml; CXCL9/Mig, 1.56 pg/ml; CXCL10/IP-10, 1.37 pg/ml; CXCL11/I-TAC, 0.55 pg/ml; and XCL1/Lptn, 1.56 pg/ml.

Stimulation of iDC with microbial compounds

Immature DC were stimulated at the concentration of 2x10^4 cells/200 μl (96-well plates, Costar) and cultured for 48 h in the absence or in the presence of the following stimuli: LPS from Escherichia coli (250 ng/ml; Sigma-Aldrich), or synthetic dsRNA poly I:C (20 μg/ml).

Determination of cytokine and chemokine production by ELISA

Determination of IL-12p70 concentrations in culture supernatants was performed by specific solid-phase sandwich ELISA as previously described. Measurements of CXCL8/IL-8 were performed by ELISA using pairs of specific mAbs and recombinant standard obtained from BioSource International (Camarillo, CA). CCL2/MCP-1 was determined using the Ab pair, rat polyclonal 20521D for coating and rabbit polyclonal 20532D for detection and recombinant CCL2/MCP-1 19781T (BD Pharmingen, San Diego, CA). CCL20/MIP-3α was determined using a DuoSet ELISA purchased by R&D Systems. The limits of detection of these ELISA are as follows: CXCL8/IL-8, 30 pg/ml; CCL2/MCP-1, 40 pg/ml; and CCL20/MIP-3α, 60 pg/ml.

RESULTS

Phenotype of DC0, DC1 and DC2

Monocyte-derived DC0, DC1, and DC2 were generated as previously described. Monocyte-derived iDC were then cultured for 48 h in the presence of TNF-α and IL-1β (maturation-inducing factors: MF) plus LPS to induce DC0, or MF+LPS+IFN-γ, or poly I:C to induce DC1, or MF+LPS+PGE2 to induce DC2 differentiation. DC maturation was comparable within the different DC groups as analyzed by the upregulation of surface expression of CD83, and the costimulatory molecules CD80 and CD86 (data not shown). To confirm whether the different effector DC subsets display the phenotype previously described, the DC0, DC1 and DC2 were stimulated for 24h with CD40L-transfected cells, thereby mimicking local engagement with T cells (Fig. 1A). DC0 and poly I:C-induced DC1 produced similar levels of bioactive IL-12p70, whereas IFN-γ-induced DC1 produced higher levels of IL-12p70. In contrast, DC2 produced low levels of IL-12p70 upon CD40 ligation.
As CCR7 is upregulated during DC maturation, the expression of this chemokine receptor was measured by real-time PCR analysis. No differences were found in CCR7 expression levels between the different DC groups (Fig. 1B).

**Figure 1.** Phenotype of DC0, DC1 and DC2 populations. Monocyte-derived iDC were cultured for 48 h in the presence of TNF-α and IL-1β (maturation-inducing factors: MF) plus LPS (DC0), or MF+LPS+IFN-γ or poly I:C (DC1), or with MF+LPS+PGE₂ (DC2). (A) The different effector DC types were stimulated for 24h with CD40L-transfected cells and IL-12p70 contents were analyzed in culture supernatants by specific ELISA. Data are expressed as mean±SD of triplicate cultures. (B) CCR7 mRNA expression levels was analyzed by real-time PCR analysis. All the DC groups expressed similar levels of CCR7 mRNA. The data are from one representative experiment out of three.

**Preferential expression of inflammatory chemokines by DC1**

Chemokines can be divided into two different groups: inflammatory and homeostatic being responsible for the movement of leukocytes under inflammatory conditions or in the steady state, respectively. Real-time PCR analysis revealed that the inflammatory chemokines CCL2/MCP-1, CCL3/MIP-1α, CCL4/MIP-1β and CXCL2/MIP-2α (Fig. 2A), were constitutively expressed by all the mature DC groups and that their expression was downregulated in response to CD40L. With the exception of CCL20/MIP-3α, which instead was upregulated upon CD40 ligation (Fig. 2A), CCL2/MCP-1, CCL3/MIP-1α, and CCL4/MIP-1β were preferentially expressed by DC0 and DC1. To verify if protein production followed the same pattern as the mRNA expression, array-based ELISAs were performed, to simultaneously measure 15 different chemokines. Fig. 2B shows that the inflammatory chemokines CCL2/MCP-1, CCL3/MIP-1α, CCL4/MIP-1β, CCL5/RANTES, CCL20/MIP-3α, CXCL1/GROα and CXCL8/IL-8 were produced constitutively by the different mature DC subsets and, with the exception of CCL2/MCP-1, their production was enhanced upon CD40 ligation. In addition, XCL1/lymphotactin production was not affected.

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upon CD40 ligation. DC2 produced lower levels of CCL2/MCP-1, CCL3/MIP-1α, CCL4/MIP-1β and CCL5/RANTES compared to DC0 and DC1, confirming the mRNA data. The pattern of CXCL1/GROα production by the different DC subsets was similar, whereas CXCL8/IL-8 production was higher in DC0 and IFN-γ-matured DC1. These data demonstrate that the expression profile of inflammatory chemokines, both at the mRNA and protein level, reflect the microenvironment of DC maturation.

**Figure 2.** DC0 and DC1 express higher levels of inflammatory chemokines. mRNA expression (A) and protein release (B) of inflammatory chemokines by DC0, DC1 and DC2 following stimulation with CD40L. mRNA levels were measured after 24 h by quantitative real-time PCR analysis and the array-based ELISA was used to measure the amount of chemokine production in culture supernatant. Protein data are expressed as mean of duplicate cultures. The data are from one representative experiment out of three.
DC0 and DC1 preferentially express CCL19/MIP-1β whereas DC2 preferentially express CCL21/6Ckine

Although, the homeostatic chemokines CCL18/DC-CK1, CCL19/MIP-3β, CCL21/6Ckine and CXCL13/BCA-1 were constitutively expressed by all the DC groups, CCL18/DC-CK1 was preferentially expressed by DC0, CCL19/MIP-3β preferentially expressed by DC0 and DC1, and CCL21/6Ckine was preferentially expressed by DC2 (Fig. 3A). CD40 ligation downregulated the expression of CCL18/DC-CK1, CCL21/6Ckine and CXCL13/BCA-1, while it upregulated the expression of CCL19/MIP-3β. Analysis of the production of CCL19/MIP-3β protein, by the different mature DC confirmed the mRNA data (Fig. 3B).

![Figure 3](image_url)

**Figure 3.** Preferential expression of CCL19/MIP-3β and CCL21/6Ckine by DC0 and DC1, and DC2 respectively. mRNA and protein release by J558-CD40L-stimulated DC0, DC1 and DC2 subsets was performed as indicated above. Protein data are expressed as mean of duplicate cultures. The data are from one representative experiment out of three.

**Preferential expression of Th1-associated chemokines by DC1**

Th1 and Th2 cells differ in their expression of chemokine receptors allowing them to migrate toward distinct types of chemokine gradients. The receptors CXCR3 and CCR5 are highly
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expressed on Th1 cells (ligands: CXCL9/Mig, CXCL10/IP-10, CXCL11/I-TAC; and CCL5/RANTES, respectively), whereas CCR3, CCR4, and CCR8 are preferentially expressed by Th2 cells (ligands: CCL11/eotaxin; CCL17/TARC and CCL22/MDC; and CCL1/I-309, respectively). Therefore, we investigated whether the profile of Th1- and Th2-associated chemokines produced by DC0, DC1, and DC2 was associated with their effector phenotype. Indeed, CXCL10/IP-10 and CXCL11/I-TAC were constitutively and preferentially expressed by both DC1 subsets. However, CXCL9/Mig was preferentially expressed by IFN-γ-matured DC1 (Fig. 4A and 4B). The Th2-associated chemokine CCL22/MDC was constitutively expressed by all the DC groups albeit at higher levels in DC2. Although the expression levels were low (pg/ml), CCL1/I-309, was preferentially produced by DC0 and IFN-γ-matured DC1. Production of CCL17/TARC was similar in all the DC groups, except for DC1 matured in the presence of IFN-γ, which produced lower levels of this chemokine. The pattern of the Th2-associated chemokines CCL1/I-309 and CCL17/TARC did not correlate with the DC effector phenotype except for CCL22/MDC (Fig. 4B). The protein data are in agreement with the mRNA expression, except for the fact that upon CD40 ligation the production of CXCL9/Mig, CXCL10/IP-10 and CXCL11/I-TAC was not affected or only marginally enhanced. Together, these data demonstrate a strict correlation of expression of Th1-associated chemokines and Th1-promoting effector DC1, while this link appears to be less stringent for Th2-associated chemokines and DC2.

Chemokine production by iDC upon stimulation with different pathogen-associated molecular patterns (PAMPs)

As effector DC1 preferentially express Th1-associated chemokines we asked whether tissue-type sentinel DC stimulated with a Th1-eliciting microbial compound, poly I:C, would lead to a similar profile of chemokine production. As shown in Fig. 5 both LPS and poly I:C were able to induce the production of the inflammatory chemokines CCL2/MCP-1 and CXCL8/IL-8 by iDC. However, we found that the production of Th1-associated chemokines CXCL9/Mig and CXCL10/IP-10 was exclusively induced in poly I:C-activated iDC. These findings confirmed the data obtained with mature DC1 (mature in the presence of LPS+MF+IFN-γ or poly I:C) which selectively produced CXCL9/Mig and CXCL10/IP-10.
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**Figure 4.** DC1 preferentially express mRNA for Th1-associated chemokines; downregulation by CD40 ligation. The Th2-associated chemokine CCL22/MDC was constitutively expressed by all the DC groups albeit at higher levels in DC2. CD40 ligation upregulated CCL22/MDC expression on all DC populations. mRNA and protein release by J558-CD40L-stimulated DC0, DC1 and DC2 subsets was performed as indicated above. Protein data are expressed as mean of duplicate cultures. The data are from one representative experiment out of three.

**Figure 5.** Chemokine production iDC upon stimulation with different microbial compounds. Monocyte-derived-iDC were stimulated for 48 h in the absence or in the presence of LPS or poly I:C and the contents of the different chemokines in culture supernatants was assessed by commercial ELISAs. Data are expressed as mean=SD of triplicate cultures. The data are from one representative experiment out of three.

**DISCUSSION**

The present study demonstrates that Th1-promoting (DC1) and Th2-promoting (DC2) effector DC constitutively express distinct patterns of inflammatory chemokines. Moreover,
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the expression of Th1-attracting chemokines is strictly restricted to the DC0 and DC1 populations. The homeostatic chemokines CCL19/MIP-3β and CCL21/6Ckine were preferentially expressed by the polarized DC1 and DC2, respectively.

DC are highly specialized APC that recognize pathogens, or their products, and mature into potent inducers of T cell immunity. A critical event in the initiation of specific immune responses is DC migration from the infected peripheral tissue to secondary lymphoid organs.\textsuperscript{11,35} In the T cell areas of LN or spleen, mature DC not only present pathogen-derived peptides to naive T cells, but also adapt the class of immune response to the type of invading pathogen by driving the development of appropriate effector Th cell subsets.\textsuperscript{36,37} To what extent chemokines produced by mature DC contribute to the development and/or maintenance of Th1 or Th2 responses remains unknown. We demonstrate that Th1-promoting effector DC (DC1) are the main producers of inflammatory chemokines such as CCL2/MCP-1, CCL3/MIP-1α, CCL4/MIP-1β and CCL5/RANTES. The production of these chemokines may sustain the recruitment of circulating CCR1-, CCR2-, CCR5- and CXCR1-positive iDC, and DC precursors to inflamed tissues important to replenish the loss of maturing DC due to their mobilization and migration to the LN.\textsuperscript{10,14} In addition, the selective expression of the CCR5 ligands CCL3/MIP-1α, CCL4/MIP-1β and CCL5/RANTES by DC1 is in agreement with the preferential attraction of CCR5+ Th1 cells by these chemokines.\textsuperscript{38} However, our data provide the first evidence that DC1 themselves may recruit Th1 effector cells to inflamed tissues. Since mature DC-derived inflammatory chemokines are downregulated upon CD40 ligation, it may point at a primary role to support ongoing Th1 responses in peripheral tissues while inflammatory chemokines should not be expressed in lymphoid organs where mature DC interact and activate naive T cells.

A prerequisite for efficient Ag presentation to naive T cells is the swift migration of mature DC into the draining LN. Effective activation of the rare Ag-specific T cell may be further promoted by the expression of T cell-attracting chemokines by the mature DC themselves once they are positioned in the T cell area of the LN. To this extent, expression of CCL19/MIP-3β and CCL21/6Ckine (CCR7 ligands) and CCL18/DC-CK1 have been detected on a fraction of DC in human LN.\textsuperscript{14,15} In agreement with this hypothesis, we could detect the homeostatic chemokines CCL18/DC-CK1, CCL19/MIP-3β, CCL21/6Ckine and CXCL13/BCA-1 in all mature DC populations analyzed. These chemokines regulate the encounters between DC, T and B cells in secondary lymphoid organs.\textsuperscript{6} To our surprise, CCL19/MIP-3β was preferentially expressed by DC0 and DC1, whereas DC2 expressed
higher levels of CCL21/6Ckine. CCR7-positive cells entering the draining LN, e.g. mature DC and naive T cells, move into the T cell area in response to CCL19/MIP-3β and CCL21/6Ckine. Our data provide evidence that production of these chemokines by mature DC may amplify the CCR7-mediated chemotactic response in an autocrine fashion. However, it is interesting to note that DC1 and DC2 take preferentially advantage of CCL19/MIP-3β and CCL21/6Ckine production, respectively.

Activated effector T cells are characterized by their responsiveness to numerous inflammatory chemokines that are produced at the site of pathogen entry and tissue injury. The expression patterns of chemokines and their receptors determine the selective recruitment of Th1 and Th2 cells. Our study demonstrates the selective production of the Th1-attracting chemokines, CXCL9/Mig, CXCL10/IP-10 and CXCL11/1-TAC, by Th1-promoting effector DC. Secretion of these chemokines by mature DC may contribute to the attraction of CXCR3+ Th1 cells to the inflammatory site prior to DC mobilization into afferent lymphatics. Alternatively, it may point at a new function for mature DC that fail to leave the inflamed tissue in sustaining the appropriate (or pathologic) polarized Th1 response in the periphery. This is corroborated by the observation that only 30% of epidermal Langerhans cells migrate to the draining LN after chemical (contact allergen), cytokine or pathogenic challenge. On the other hand, upregulation of expression of these chemokines in response to CD40 ligation may also support the DC:T-cell interaction in lymphoid organs. Indeed, it was demonstrated that DC-derived CXCL10/IP-10 regulates DC-Th1 cell cluster formation and retention of CD4+ T lymphocytes during their polarization towards Th1 in the T cell areas of draining LN. This concept of Th1 retention may provide a new insight into the mechanism of polarized effector T cell generation. In addition, the data obtained with iDC stimulated with different microbial compounds confirm the notion that the preferential production of the Th1-associated chemokines CXCL9/Mig and CXCL10/IP-10 is associated with a Th1-eliciting microbial compound, namely poly I:C.

In contrast to chemokines linked to Th1 immunity, the expression of Th2-associated chemokines was not strictly associated with the Th2-promoting effector DC phenotype. Except for CCL22/MDC that was preferentially and constitutively expressed by mature DC2. In agreement with previous reports, its expression was upregulated upon CD40 ligation. CCL22/MDC-expressing DC with a mature phenotype have been detected in situ in peripheral tissues, such as the skin. Together with our data these studies suggest a role for mature DC2-derived chemokines in maintaining Th2 responses locally in the periphery.
The current findings suggest that diverse DC populations produce different repertoires of homeostatic and inflammatory chemokines. The production of inflammatory chemokines by mature DC, and its downregulation upon CD40 ligation, led us to propose a novel role for mature DC that are present in peripheral tissues in recruiting appropriate effector leukocyte populations to the site of pathogen entry.

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