PGE₂ transiently enhances DC expression of CCR7 but inhibits the ability of DCs to produce CCL19 and attract naïve T cells

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Abstract

Prostaglandin E$_2$ (PGE$_2$) is an inflammatory mediator often used to increase CCR7 expression in the dendritic cells (DC) used as cancer vaccines and to enhance their responsiveness to lymph-node-associated chemokines. Here, we show that high surface expression of CCR7 on PGE$_2$-matured DCs is associated with their suppressed production of the endogenous CCR7-ligand, CCL19, and is reversible by exogenous CCL19. In contrast to the PGE$_2$-matured DCs, DCs matured in the presence of TLR-ligands and interferons produce high levels of both CCL19 and CCR7 mRNA/protein, but show selectively-reduced expression of surface CCR7, which is compensated after DC removal from the CCL19-rich maturation environment. In accordance with these findings, PGE$_2$-matured DCs show significantly-higher in vitro migratory responsiveness to lymph node-associated chemokines directly after DC generation, but not after additional short-term culture in vitro, nor in vivo in patients injected with $^{111}$In-labelled DCs. The differences in CCL19-producing ability imprinted during DC maturation result in their different abilities to attract CCR7$^+$ naïve T cells. Our data help to explain the impact of PGE$_2$ on CCR7 expression in maturing DC and demonstrate a novel mechanism of regulatory activity of PGE$_2$, mediated by the inhibition of DC’s ability to attract naïve T cells.
Introduction

Prostaglandin E2 (PGE2) is an inflammatory mediator with suppressive activity at several levels of the immune response\textsuperscript{1-3}. PGE\textsubscript{2} selectively impairs the production of IL-2 and IFN\(\gamma\) in T cells\textsuperscript{4,5} inhibits the responsiveness to T cell-activating and Th1-driving cytokines such as IL-2 and IL-12p70\textsuperscript{6,7}, blocks the production of DC-produced pro-inflammatory cytokines, including IL-12p70\textsuperscript{8-10}, and induces the production of IL-12R antagonist IL-12p40 homodimer\textsuperscript{11,12}. Recently, PGE\textsubscript{2} has been also shown to promote the ability of DCs to preferentially attract the inhibitory T(reg) subset of CD4\textsuperscript{+} T cells\textsuperscript{13} and to directly promote the development of Tregs\textsuperscript{14,15}.

In apparent contrast to these suppressive functions, PGE\textsubscript{2} also has been reported to synergize with tumor necrosis factor-alpha (TNF\(\alpha\)) in the induction of DC maturation\textsuperscript{10,16}, and in promoting CCR7 expression and the chemotactic responsiveness of DCs to CCL19 and CCL21\textsuperscript{17-19}, the two CCR7 ligands known to promote DC entry into lymph nodes\textsuperscript{20-22}. These observations opened the possibility that PGE\textsubscript{2} may also support the induction of antigen-specific immune responses by promoting the migration of Ag-carrying DCs to the draining lymph nodes and their interaction with lymph-node-based naïve and central memory T cells. Based on these observations, PGE\textsubscript{2} is frequently included in the cytokine cocktails\textsuperscript{16} used to induce mature DCs for clinical use as vaccines against cancer\textsuperscript{23}.

Taking into account these apparently paradoxical effects of PGE\textsubscript{2} on DC functions, and guided by the observations from other cell systems that the surface levels of chemokine receptors can be regulated by their chemokine ligands\textsuperscript{24-27}, we tested the impact of PGE\textsubscript{2} on the regulation of the CCR7-CCL19/21 system in maturing DCs.
Materials and methods

Media and reagents
Serum-free CellGro DC medium (CellGenix, Freiburg, Germany) was used for the DC cultures. The following factors were used to generate mature DC: Recombinant human (rhu) granulocyte macrophage colony-stimulating factor (GM-CSF) and IL-4 (gifts from Schering-Plough, Kenilworth, NJ); IFN-α (Intron A- IFN-α-2b, Schering-Plough); rhuTNF α, rhuIL-1β, and rhuIFN-γ (Strathmann Biotech, Hannover, Germany); rhuIL-6 (Genzyme Cambridge, MA); PGE₂ and poly-I:C (both from Sigma, St. Louis, MO). CD40L-transfected J558 cells 28 (gift from Dr. P. Lane, University of Birmingham, United Kingdom) were used as an equivalent to activated CD4⁺ T cells 28,29. Recombinant CCL19 and CCL21 were purchased from Peprotech, Rocky Hill, NJ. FITC-labeled CCR7-specific antibody for flow cytometry (clone 150503, R&D Systems, Minneapolis, MN) and the relevant isotype control (IgG2a) used for FACS staining were obtained from R&D systems. CCR7 blocking antibody (3D12) was purchased from BD Biosciences, San Jose, CA.

DC cultures
Peripheral blood mononuclear cells (PBMCs) obtained from healthy donors were isolated with lymphocyte separation medium (CellGro Mediatech, Herndon, VA). Monocytes were isolated on density gradients, using Percoll (Sigma, St. Louis, MO) or Isolate (Irving Scientific, Santa Ana, CA) followed by plastic adherence, as described 29,30. Monocytes were cultured for 6 days in 24-well plates (Falcon, Becton Dickinson Labware, Franklin Lakes, NJ) at 5 x 10⁵ cells per well in rhu GM-CSF and IL-4 (both 1,000 IU/mL). On day 6, DC maturation (48 hours) was induced using the indicated combinations of the following factors: IL-1β (25 ng/mL); TNFα (50 ng/mL), IFNγ (1,000 units/mL); IL-6 (1,000 units/mL); PGE₂ (10⁻⁶ M); Poly-I:C (20 μg/mL); IFNα (3,000 units/mL) 30.
**Isolation of naïve CD4+ T cells**

Mononuclear cells from peripheral blood of healthy donors were isolated on density gradient using Lymphocyte Separation Medium (CellGro, Mediatech). Naïve CD4+ T cells were isolated by negative magnetic selection using the EasySep® human CD4+ T cell enrichment kit (StemCell Technologies Inc., Vancouver, BC, Canada) according to the manufacturer’s protocol.

**Analysis of CCL19 and CCL21 production by Taqman and ELISA**

Total RNA was extracted using Qiagen RNAeasy kit (Qiagen, Valencia, CA) according to manufacturer’s protocol. cDNA synthesis was performed on 2 μg of extracted RNA in 20 μL reaction volume using Retroscript kit (Ambion, Austin, TX) according to the manufacturer’s protocol. Real-time analysis was performed on 25 ng of sample cDNA using pre-made CCR7, CCL19, CCL21 specific Taqman primers and probes from Applied Biosystems (Foster City, CA), in 25 μL reaction volume using universal Taqman kit with UNG (Applied Biosystems, Foster City, CA) following manufacturer’s protocol. Samples were analyzed with an ABI Prism 7700 sequence analyzer (Applied Biosystems, Foster City, CA). The expression of each gene was normalized to HPRT1 housekeeping gene and expressed as fold increase ($2^{-\Delta CT}$), where $\Delta CT = [CT_{\text{Target gene}}] - [CT_{\text{HPRT1}}]$. Supernatants from DC cultures (20 x 10^3 DC/0.2 ml, when indicated stimulated with 50 x 10^3 CD40L-expressing J558 cells) were analyzed for CCL19 protein concentration by indirect sandwich ELISA, using primary and secondary antibodies from Peprotech (Rocky Hill, NJ). In brief, ELISA plates (Corning Inc, Corning, NY) were coated overnight at room temperature with the primary antibody (10 μg/mL), followed by washing and blocking with PBS + 4% BSA for 1 hour. The samples were added to the wells and incubated for 1hr and subsequently washed and incubated with the biotinylated secondary antibody (2.5 μg/mL) for 1 hour. The plates were washed and incubated for 30 minutes with Streptavidin-HRP conjugate (Pierce Biotechnology Inc, Rockford, IL), diluted 1:8000 in wash buffer.
(50 mM Tris, 0.2% Tween). The plates were washed and detected with 50 µL of TMB substrate (Pierce). Reactions were stopped with 2% H2SO4 and absorbance at 450nm was measured.

**Analysis of surface and intracellular CCR7 levels**

FITC-labeled CCR7 antibody (Clone 150503; R&D Systems) was used for surface and intracellular CCR7 staining. For intracellular staining, the cells were permeabilized with Permiflow (Invirion, Inc., Frankfort, Michigan) reagent and then stained with CCR7 antibody.

**Analysis of *in vitro* chemotaxis**

DC chemotaxis assays were performed in 96 transwell plates with 5µm pore size polycarbonate filter (Corning Inc, Corning, NY). The lower chamber was filled with 200 µL of recombinant human CCL21 in Cellgenix DC medium (Cellgenix). 50 x 10^3 DCs in 50µL were added to the upper chamber and the migration chambers were incubated for 2 hours at 37°C. To analyze chemotaxis of naïve CD4^+^ T cells, 5.0 µm pore size 24 well transwell plates from the same company were used. 200 x 10^3 cells) of naïve CD4 T cells (in 100µL) were allowed to migrate for 3 hrs towards DC culture supernatants (250 x 10^3 DC/0.5 ml, stimulated for 24 hours with 250 x 10^3 CD40L-expressing J558 cells). When indicated, in order to block the CCR7-dependent component of migration, naïve CD4^+^ T cells were treated for 30 minutes with anti-CCR7 blocking antibody (3D12; BD Biosciences; 20 µg/ml), prior to chemotaxis.

**Analysis of DC migration in vivo in melanoma patients:**

The clinical trial was approved by the ethics committee of the University of Heidelberg. Patients with stage IIIc-IV melanoma were eligible. DCs for clinical use were generated under cGMP conditions from autologous monocytes obtained by leukapheresis. Monocytes were selected using CD14 microbeads (CliniMACS, Miltenyi Biotech, Bergisch Gladbach, Germany) and cultured in CellGro
DC medium (CellGenix) supplemented with GM-CSF (800 IU/ml, Novartis, Nurnberg, Germany) and IL-4 (1000 IU/ml, CellGenix, Freiburg, Germany) for 6 days. On day 6 maturation was induced by either (for sDCs) TNF-α (5 ng/ml, Beromun, a gift from Boehringer-Ingelheim), IL-1β (2 ng/ml, CellGenix), IL-6 (5 ng/ml, CellGenix), and PGE2 (1 µg/ml; Dinoproston, Pfizer, Berlin, Germany) or (for αDC1s) a cocktail of TNF-α (50 ng/ml), IL-1β (25 ng/ml), IFN-γ 1000 U/ml (Immukin, Boehringer-Ingelheim, Biberach, Germany) INF-α2a (1000 U/ml; Roferon A, Roche, Germany), and Poly I:C (20 µg/ml; Sigma-Aldrich, Seelze, Germany). DCs were labeled with (1.4 MBq) 111indium-oxinate (Tyco Healthcare, Neustadt, Germany) for 15 minutes and washed with PBS/1% human serum albumin. The labeling efficiency was 71.8±6.4% with viability of more than 95% at 24 h after the labeling procedure. One million 111In-labeled DC were injected intradermally on both upper thighs (αDC1s: right leg; sDCs: left leg), 10 cm distal to the inguinal lymph nodes. Migration was followed by scintigraphic imaging with a SPECT/CT camera (Hawkeye, GE Healthcare, Munich, Germany) directly after injection, and 1, 24, and 48 hours after injection. We performed whole body scans images for 20 minutes (10cm/min) and single planar scans of the pelvis for 10 minutes. Activity that accumulated in lymph nodes was quantified by region of interest analysis.

**Statistical analysis:**

Chemokine dose-responsiveness of DCs in vitro was analyzed by ANOVA, while the in vivo migration was analyzed by Wilcoxon’s matched-pair test. In all other cases, the data were evaluated using Student’s t- test (two-tailed), with p <0.05 considered as significant.
Results:

PGE$_2$-matured “standard” (s)DC and αDC1s show transient differences in CCR7 expression and CCR7 responsiveness in vitro, but similar lymph node-migratory function in vivo. PGE$_2$-matured “standard” (s)DC and αDC1 are two types of mature DCs that can be generated in clinically-applicable serum free conditions. While the PGE$_2$-based protocols of DC maturation have been shown to be associated with suppression of IL-12p70 production $^{10-12,30}$, the ability of PGE$_2$ to enhance the DC expression of CCR7 and in vitro migratory responsiveness to CCL19 and CCL21 $^{17-19}$ (CCR7 ligands guiding DCs to lymph nodes $^{20-22,31}$) led to the application of PGE$_2$ in inducing mature “standard” DCs for clinical applications $^{16,23}$. In contrast to such IL-1β/TNFα/IL-6/PGE$_2$-induced “standard” (s)DCs, mature type-1-polarized DCs obtained in the presence of TLR-ligands and interferons $^{30,32-34}$ (including IL-1β/TNFα/IFNα/IFNγ/poly-I:C-induced, type-1-polarized DCs; αDC1) $^{30}$, are capable of producing much higher levels of IL-12 and inducing tumor-specific CTLs in vitro $^{30,33,35}$, but show lower in vitro migratory responsiveness to CCR7-binding chemokines $^{30}$.

In accordance with previous observations $^{30,35}$, PGE$_2$-matured DCs showed statistically higher surface CCR7 expression than αDC1s (Figure 1A, left), and elevated ability to migrate towards CCL21, the lymph-node-associated CCR7 ligand (Figure 1A, right), despite significant variability of the magnitude of such differences observed in DC preparations from different donors. However, even the most profound differences in CCR7 expression on sDCs and αDC1s observed in some donors (Figure 1B, left) were rapidly compensated following the harvesting of DCs from the maturation cultures and re-plating them in neutral conditions in the presence of GM-CSF only (Figure 1B, right). This compensation of the differences in surface CCR7 expression was reflected by the elimination of differences in migratory capacity in vitro (Figure 1C).
In accord with the ability of the differentially-matured DCs to compensate for the maturation-associated differences in CCR7 expression and responsiveness to lymph-node-produced CCR7 ligands) also in vivo, sDCs and αDC1s showed similar ability to migrate to the draining lymph nodes, following their intradermal injection (Figure 1D; αDC1s: right leg; sDCs: left leg). We observed retention of the radioactivity at the injection site of 79±4% and 55±2% after 24 hours and 48 hours, respectively, with nodal accumulation starting to be detectable at 24 hours and detectable in all cases at 48 hours. Similar to earlier studies, the overall migratory effectiveness of all DCs was within the range of 0.69% to 4.6% with a single preparation migrating with 9% effectiveness (Figure 1D). In four individuals, the migration of sDCs (range: 0.69% to 9.1%) was higher than the migration of αDC1s (range: 1.1% to 3.7%), while αDC1s were superior in two individuals of the six tested.

Selectively-enhanced surface CCR7 expression on PGE₂-matured DCs occurs despite reduced levels of CCR7 gene expression and is abrogated by exogenous CCL19. In an attempt to address the mechanism of the transient differences in CCR7 expression between sDCs and αDC1s, we compared surface and intracellular CCR7 protein levels and the expression of CCR7 mRNA. Unexpectedly, even in the donors showing the most pronounced differences in surface CCR7 expression (Figure 2A, left), we could not detect such differences when analyzing the total CCR7 protein levels in permeabilized DCs (Figure 2A, Middle). Even more strikingly, the PGE₂-matured sDC, expressed substantially reduced, rather than enhanced, levels of CCR7 mRNA (Figure 2A, right).

Prompted by this dissociation between the levels of surface CCR7 protein and CCR7 mRNA expression and by previous reports showing that surface levels of chemokine receptors can be affected by ligand-induced internalization 24-27, we tested whether the differences in surface expression of CCR7 between DCs and αDC1s can be abolished by exogenous CCR7 ligands. In accordance with previous reports 24-27, we could not detect any impact of CCL21 on surface CCR7 expression.
(Supplemental Figure 1). However, the addition of CCL19, the only DC-produced CCR7 ligand (see below) strongly reduced the surface levels of CCR7 expression of sDCs (Figure 2B, Top). In contrast to its impact on surface expression of CCR7, CCL19 treatment did not affect the overall levels of CCR7 in sDCs (or αDC1s; Figure 2B, Bottom).

PGE₂-matured DCs show suppressed production of CCL19: PGE₂ is a powerful inhibitor of CCL19 production by human DC

Guided by the selectively enhanced expression of surface CCR7 (but not total cell-associated CCR7 protein or CCR7 mRNA) in PGE₂-matured DCs and by the ability of exogenous CCL19 to abrogate that advantage, we compared the ability of PGE₂ to affect the production of endogenous CCR7 ligands by human DCs. In accordance with the possibility that the differences in endogenous CCL19 production may affect the rate of internalization of CCR7, we observed high CCL19 protein secretion and high expression of CCL19 mRNA selectively in αDC1s, but not sDC (Figure 3A, and Supplemental Figure 2). In accordance with the in vivo observations that lymphatic endothelium, rather than DC, are the source of CCL21, we did not detect CCL21 expression in either DC type (data not shown).

Our in-depth analysis of the contribution of the individual DC-maturation-inducing factors to the differential regulation of CCL19 production in sDCs and αDC1s, identified TNFα, IFNα and poly-I:C (TLR3-ligand), as the key CCL19-inducing factors, with PGE₂ playing a dominant inhibitory role, suppressing the CCL19 mRNA transcription (Supplemental Figure 2) and CCL19 protein secretion (Figure 3B).

PGE₂ also showed a dominant inhibitory role, suppressing the CCL19 production induced by another TLR ligand, LPS (Figure 3B and Supplemental Figure 2).
Conditions of maturation determine the ability of mature DCs to produce CCL19 after subsequent (re)activation

In order to assess the stability of the maturation-induced differences in CCL19 production between the DCs matured in the absence and presence of PGE2, we analyzed the production of this factor during DC maturation (during direct exposure to interferons and TLR-Ls, or PGE2) and after DC removal from these different maturation environments and upon their further culture in the absence or presence of CD40L, used as a model of interaction the differentially-matured DCs with CD40L-expressing CD4+ T cells.28,29.

We observed that while the difference in CCL19 production between the DCs maturing in the absence or presence of PGE2 was reduced after removal of DCs from the maturation cultures, it was restored after subsequent DC reactivation (Figure 3C). The enhanced CCL19 production in αDC1s was reduced in the DCs harvested and re-cultured for 24 hours in the absence of maturation-inducing factors, but was restored to similarly high or higher levels after DC harvesting and subsequent CD40L stimulation. In contrast to αDC1s, isolated sDCs failed to produce significant amounts of CCL19 either spontaneously or after subsequent stimulation with CD40L (Figure 3C), despite the absence of PGE2 at this stage. These data indicate that the conditions of DC maturation prime DC for subsequent ability to secrete CCL19 in neutral environments and suggest that DC matured in different conditions may have different abilities to interact with CCL19-sensitive CCR7-expressing T cells (such as naïve and central memory T cells) after reaching the draining lymph nodes.

PGE2-matured DC show impaired ability to attract naïve T cells

In order to verify whether the CCL19 produced by αDC1 is indeed functional, we evaluated the ability of αDC1s and sDCs to attract CCR7-expressing naïve CD4+ T cells. The contribution of the CCR7 pathway was determined using CCR7 blocking antibodies. As shown in Figure 4, while high numbers of naïve CD4+ T cells migrated towards αDC1 supernatants, sDCs were completely
unable to increase the migration of naïve T cells above the background observed with medium alone. As expected, the migration of naïve T cells was completely abolished in the presence of CCR7 blocking antibody.
Discussion:

Our data demonstrate a novel function of a chronic-inflammatory mediator, PGE₂, as a powerful inhibitor of DC production of CCL19, the key chemokine attracting naïve and central-memory T cells. They show that PGE₂ is a dominant inhibitor of the CCL19 production induced by TNFα, interferons, or TLR-Ls (TLR3 ligand poly-I:C or TLR4 ligand, LPS; see Figure 3B).

The stability of the maturation-induced ability to produce CCL19 indicate that the conditions of DC maturation in peripheral tissues may affect their ability to interact with naïve or central memory T cell subsets within the lymph nodes. Although the elevated CCL19 production by the DCs maturing in the presence of TNFα, interferons and TLR-Ls (rather than PGE₂) is rapidly terminated after DCs leave the presence of interferon- and TLR-L-dominated environment, Ag-carrying DC1 are highly likely to receive secondary chemokine-inducing signals after reaching the lymph nodes from CD40L-expressing Th cells, resulting in a second wave of CCL19 production by interferon- and TLR-L-primed DCs (see Figure 3C) and additional recruitment of naïve and central memory T cells.

The current data helps to understand the complex role of PGE₂ in the regulation of antigen-specific immune responses and the paradoxical effects of PGE₂ on DC functions. PGE₂ is an inflammatory mediator over-expressed in multiple inflammatory states, but prevalent at late stages of inflammatory reactions and in chronic inflammation ⁰⁻³. PGE₂ was shown to suppress T cell proliferation and their production of key proinflammatory and antitumor cytokines in direct and indirect manner ⁴⁻¹², as well as to promote the interaction of DCs with Tregs ¹³ and to directly promote Treg development ¹⁴,¹⁵. Paradoxically however, the ability of PGE₂ to promote the in vitro chemotactic responsiveness of DCs to CCL19 and CCL21 ¹⁷⁻¹⁹, the two CCR7 ligands directing activated DCs into lymph nodes ²⁰⁻²², suggested that PGE₂ may also support antigen-specific immune responses and led to applications of PGE₂-based cytokine cocktails in preparation of DC-based vaccines.
The current results help to reconcile these paradoxical observations and to explain the ability of PGE2 to enhance the expression of CCR7 on the surface of maturing DCs. We observed that the enhanced CCR7 expression on the surface of PGE2-matured DCs occurs despite their reduced, rather than enhanced, expression of CCR7 mRNA and it is reversed by exogenous CCL19, the CCR7 ligand known to induce CCR7 internalization\(^\text{24-27}\) that is selectively produced by the DCs matured in the absence of PGE2. These data, jointly with the lack of differences at the level of total (surface plus intracellular) CCR7 protein expression, argue that the suppressed production of CCL19, preventing ligand-induced CCR7 internalization, is the key factor responsible for enhanced surface CCR7 expression in DCs maturing in the presence of PGE2.

Importantly, the differences in surface CCR7 expression between the DCs maturing in the absence or presence of PGE2 are rapidly compensated following DC removal from the maturation cultures, and after the cessation of high-level production of endogenous CCL19 in DCs matured in the absence of PGE2. The reduced *in vitro* migratory function of DCs maturing in the absence of PGE2 was clearly evident directly after DC removal from the CCL19-rich maturation cultures, but reduced or eliminated following additional culture of DCs in the absence of CCL19 (*Figure 3*). In accordance with these observations, our current *in vivo* data (*Figure 1D*) also did not demonstrate an advantage of PGE2-matured DCs in lymph-node homing. However, they do not preclude the possibility that such an advantage can be demonstrated in larger studies. In either case, our current observations suggest that any potential advantage of PGE2 in promoting DC migration needs to be balanced against its negative impact on the CCL19 production by DCs.

The ability of PGE2, the predominant inflammatory mediator during late stages of inflammation, to program local DCs for preferential interaction with T\(_\text{reg}\)s with the concomitant inhibition of their ability to attract type-1 immune effector cells\(^\text{13}\), and naïve and central memory T cells (the current data) helps to explain the dominance of the suppressive effects at later stage of
immune responses and the state of generalized immunosuppression associated with chronic inflammation and cancer, facilitating the design of effective therapies.

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**Authors’ Contributions:**

R.M. Designed the laboratory studies, performed the experiments and analyzed the data, and wrote the manuscript; J. M-B. designed the clinical studies and analyzed the data; U.H. designed and supervised the clinical studies; T.A.R. Helped to design the studies, analyse the data and participated in preparation of the manuscript. D.S. designed the studies, supervised the performance and data analysis of clinical aspects, and wrote the manuscript; P. K. designed the studies, supervised the performance and data analysis of the laboratory aspects, and wrote the manuscript.

**Conflicts of Interests:**

Alpha-type-1 polarized DCs (αDC1) \(^{30}\) used in this paper, are a topic of a pending patent application. None of the authors receives any form of support or remuneration related to αDC1s or other aspects of this study.
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Figure legends:

Figure 1. Transient elevation of CCR7 expression and in vitro migratory responsiveness to lymph node-associated chemokines in PGE$_2$-matured DCs. (A, left) Surface CCR7 expression in DCs matured into αDC1s or sDCs in 9 different donors. (A, right) In vitro migratory response of αDC1s and sDCs to CCL21, a secondary-lymphoid organ chemokine (data from 6 different donors shown as mean ± SD). (B) Surface expression of CCR7 protein on αDC1s and sDCs at 0 (left) and 24 hours after completion of maturation and re-plating in the absence of the maturation-inducing factors (right). (C) In vitro migratory response to CCL21 of αDC1s and sDCs, directly (left) and 24 hours (right) after removal from the maturation cultures. The numbers in gray boxes represent the ratios between the numbers of sDCs and αDC1 that migrated to each individual concentration of CCL21. Similar data were obtained in two independent experiments. (D, left) In vivo migration of αDC1s and sDCs to the lymph nodes. Scintigraphic image of $^{111}$Indium labeled αDC1 and sDC at intra-dermal injection site and draining lymph nodes at 48 hours in a single representative patient. (D, right) In vivo migration of αDC1s or sDCs in 6 patients (each pair of dots represents each individual patient).

Figure 2. Selectively-elevated CCR7 expression on the surface of PGE$_2$-matured DCs occurs despite reduced levels of CCR7 mRNA and is suppressed by the exposure to CCL19. (A) Directly after harvesting from maturation cultures, αDC1s and sDCs were analyzed for the expression of surface CCR7 (left panel) and total (surface and intracellular; middle panel) CCR7 protein, and for CCR7 mRNA expression (right panel). (B) Surface (top) and total (bottom) expression of CCR7 protein was analyzed in αDC1s and sDCs matured in the absence or presence of exogenous CCL19 (100ng/ml). Similar data were obtained in three independent experiments (3 different donors).
Figure 3. **PGE2 suppresses the production of endogenous CCL19 in DCs induced to mature by TNFα, IFNα, or TLR ligands; Poly-I:C or LPS: Stability of the maturation-imprinted ability to produce CCL19.** (A) CCL19 mRNA (*left*) and CCL19 protein expression (*right*) in αDC1s and sDCs. Representative data from one of six different donors. (B) CCL19 secretion by DCs exposed for 48 hrs to IFNα or PGE2 (or both) in absence or presence of maturation factors, TNFα, Poly-I:C (TLR3-ligand) or LPS (TLR4 ligand. Cumulative data (mean ± SE) from 3 different donors. (C) CCL19 levels in αDC1s or sDCs during maturation or after maturation with or without CD40L. Cumulative data (mean ± SE) from 3 different donors.

Figure 4. **PGE2-matured DCs show suppressed ability to attract naïve CD4⁺ T cells.**

Negatively-isolated naïve CD4⁺ T cells were allowed to migrate towards 24 hour culture supernatants from αDC1s and sDCs in transwell migration chambers (3 hours), in the absence (white bars) or presence (black bars) of CCR7-blocking antibody. The migrated T cells were collected from the bottom chamber and counted. The dotted line represents the average spontaneous migration of T cells in the absence of DC supernatants. Cumulative data (mean ± SE) from four different donors.
Figure 1
Figure 2
Figure 3
Figure 4
PGE₂ transiently enhances DC expression of CCR7 but inhibits the ability of DCs to produce CCL19 and attract naive T cells

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