

Toll-like receptor agonists differentially regulate cysteinyl-leukotriene receptor 1 expression and function in human dendritic cells

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Background: Dendritic cells (DCs) acquire, during their maturation, the expression of the chemokine receptor CCR7 and the ability to migrate to lymph nodes in response to CC chemokine ligand 19 (CCL19). This migration is impaired in mice lacking the leukotriene (LT) C₄ transporter and restored by addition of exogenous LTC₄.

Objective: To define the role of LT in human DC function, we studied the expression and function of the cysteinyl-leukotriene (CysLT) receptors during DC differentiation from monocytes and subsequent maturation.

Methods: Receptor expression was measured by flow cytometry and real-time PCR. Responsiveness to LTD₄ stimulation was assessed by calcium flux and chemotaxis.

Results: Maturation of DC with LPS, a classic Toll-like receptor 4 agonist, reduced CysLT receptor 1 (CysLT1) expression by 50%, whereas CysLT receptor 2 expression was increased. In contrast, the Toll-like receptor 3 agonist poly I:C and cytidylic acid (polyI:C) had no effect on receptor expression. Downregulation of CysLT1 expression by LPS could not be mimicked by TNF- α alone or in combination with IL-1 β or IL-6. It was, however, prevented by inhibitors of COX and could be reproduced by a combination of TNF- α and prostaglandin E₂. Immature DCs and DCs matured with polyI:C, but not with LPS, responded to LTD₄ with a robust cytosolic calcium flux, which was prevented by the CysLT1 antagonist montelukast. LTD₄ induced DC chemotaxis and enhanced DC migration in response to CCL19 in DCs matured with polyI:C, but only weakly in DCs matured with LPS.

Conclusion: Our data suggest that human DCs may differentially respond to leukotriene, depending on their maturational stimuli.

Clinical implications: Our study demonstrates that some microbial agents can reduce the migration of dendritic cells

in response to leukotrienes, with potential for differential involvement of these cells in allergic inflammation. (*J Allergy Clin Immunol* 2006;117:1155-62.)

Key words: Human, dendritic cells, lipid mediators, leukotriene, allergy, inflammation

Dendritic cells (DCs) are highly efficient antigen-presenting cells that play a major role in the regulation of immune responses to antigens.¹⁻³ They capture antigens at the periphery of the body and transport them to lymphoid organs. Antigen uptake occurs via constitutive endocytosis, pinocytosis, or phagocytosis, and the ability of DCs to take up antigen is related to their development and inversely correlated with their maturational state.^{4,5} Immature DCs (iDCs) efficiently capture antigen but express modest amounts of MHC class II and costimulatory (CD40, CD80, CD86) molecules at the cell surface. After maturation, DCs express high levels of MHC II and costimulatory molecules, but capture antigen poorly. They are, at that stage, best equipped to present antigen incorporated during their immature stage to T cells.

During their development, DCs differentially regulate their expression of chemokine receptors to facilitate their initial migration to antigen-exposed tissues and, at a later stage, to allow their migration from these tissues to regional lymph nodes.^{6,7} Whereas iDCs express a variety of chemokine (CCR1, CCR2, CCR5, CXCR1) and other (C5a, fMLP) receptors that may contribute to their attraction to mucosal sites,⁸ mature DCs (mDCs) express CXCR4 and particularly CCR7, which renders them responsive to the chemokines CCL19 (MIP3 β) and CCL21 and allows their migration to the T-cell areas of lymph nodes.^{6,7} Because mDCs from CCR7 knockout mice cannot migrate to lymph nodes, it appears that the interaction between CCR7 and its ligands is essential for normal migration of mDCs from periphery into the regional lymph nodes.

Mature DC migration in response to CCL19 is impaired in mice lacking the multidrug transporter ATP-binding cassette c1 (formerly known as multidrug resistant protein 1 [MRP-1]), which actively transports the lipid mediator cysteinyl-leukotriene (LT) C₄ out of the cell.^{9,10} Absence of ATP-binding cassette c1 leads to intracellular accumulation of LTC₄ and no conversion to LTD₄ by the extracellular γ -glutamyl transpeptidase.¹¹ Although addition of

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Abbreviations used

cysLT:	Cysteinyl-leukotriene
CysLT1:	Cysteinyl-leukotriene receptor 1
CysLT2:	Cysteinyl-leukotriene receptor 2
DC:	Dendritic cell
FITC:	Fluorescein isothiocyanate
iDC:	Immature dendritic cell
LT:	Leukotriene
mDC:	Mature dendritic cell
MoDC:	Monocyte-derived dendritic cell
MIP3 β :	Macrophage inflammatory protein 3 β
PBML:	Peripheral blood mononuclear leukocyte
PG:	Prostaglandin
polyI:C:	Poly inosinic and cytidylic acid
TLR:	Toll-like receptor

exogenous LTC₄ restores murine DC chemotaxis to CCL19,⁹ the mechanism through which LTC₄ or LTD₄ exert their effects on DC migration, or other functions, is unknown. Recently, Machida et al¹² reported that cysteinyl-leukotrienes increased IL-10 production by allergen-pulsed mouse myeloid DC and enhanced their capacity to induce lung eosinophilia and IL-5 production in a murine asthma model.

Cysteinyl-leukotrienes (cysLT) act through 2 G-protein-coupled receptors, designated cysteinyl-leukotriene receptor 1 (CysLT1) and cysteinyl-leukotriene receptor 2 (CysLT2), that display a different but partially overlapping tissue distribution.¹³⁻¹⁵ CysLT1 has higher affinity for LTD₄ than for LTC₄ and is expressed mainly in bronchial smooth muscle and in most leukocyte populations.¹⁶ CysLT2 has equal affinity for LTC₄ and LTD₄, and is widely expressed in many tissues, including brain, adrenals, lung, spleen, endothelium, and leukocytes.¹⁵ Leukotriene receptor expression in human DCs has not been examined.

In the current study, we investigated the effects of LTC₄ and LTD₄ on the phenotype and functions of human monocyte-derived DCs (MoDCs). In particular, we analyzed the differential expression of CysLT1 and CysLT2 in human MoDCs during their differentiation and maturation, as well as the modulation by leukotriene of their migratory capacity in response to CCL19.

METHODS

Reagents

Human recombinant GM-CSF, IL-1, IL-4, IL-6, IL-15, MIP-3 β (CCL19), TGF- β , and TNF- α were purchased from PeproTech (Ottawa, Ontario, Canada). Rabbit polyclonal antihuman CysLT1R and CysLT2R Abs were obtained from Cayman Chemical (Ann Arbor, Mich); rabbit IgG isotype control was obtained from Southern Biotechnology Associates (Birmingham, Ala); mouse IgG isotype control (G155-178), anti-CD83 (HB15e), anti-CD86 (2331 [FUN-1]), CD1a-fluorescein isothiocyanate (FITC; HI149), CD14, and human leukocyte antigen (HLA)-DR were purchased from Becton Dickinson (BD Pharmingen, Mississauga, Ontario, Canada); FITC-conjugated goat antirabbit IgG and FITC-conjugated goat anti-mouse IgG were obtained from Bio/Can Scientific (Mississauga,

Ontario, Canada). Indomethacin, the COX-2 inhibitor NS-398, LTD₄, and LTC₄ were obtained from Cayman Chemical. The CysLT1 selective antagonist montelukast was a generous gift from Merck-Frosst (Pointe-Claire, Québec, Canada). RPMI 1640 and nonessential amino acids were purchased from Gibco (Burlington, Ontario, Canada), and AIM-V medium was from Invitrogen (Burlington, Ontario, Canada). FBS, lipid-free BSA, LPS from *Escherichia coli* serotype 0127:B8, LPS from *Serratia marcescens*, LPS from *Salmonella minnesota*, prostaglandin (PG) E₂, poly inosinic and cytidylic acid (polyI:C), and zymosan A from *Saccharomyces cerevisiae* were purchased from Sigma-Aldrich (Oakville, Ontario, Canada). The cysLT receptor antagonists Bay-u9773 and Ly171863 were obtained from Biomol Research Laboratories (Plymouth Meeting, Pa).

Generation of MoDCs

Monocyte-derived DCs were generated from human peripheral blood mononuclear leukocytes (PBMLs) obtained from normal donors after informed consent in accordance with an Internal Review Board-approved protocol. PBMLs were separated by standard density gradient centrifugation on Ficoll-Paque (Amersham Biosciences, Baie d'Urfé, Quebec, Canada), as previously described.¹⁷ PBMLs were then resuspended at 4×10^6 cells/mL in RPMI 1640 medium supplemented with 10% heat-inactivated FBS, 100 U/mL penicillin, and 100 ng/mL streptomycin and allowed to adhere to plastic Petri dishes coated with defibrinated autologous serum for 1 hour at 37°C. Nonadherent cells were removed by rinsing with PBS, and adherent cells (>90% monocytes) were detached with EDTA (0.01 mol/L) in RPMI 1640. Mononuclear cells were further purified with anti-CD14 mAb-coated MicroBeads using MACS CD14 isolation kits from Miltenyi Biotec (Auburn, Calif), according to the manufacturer's instructions. Purified CD14⁺ cells were cultured in this medium, supplemented with 2 mmol/L L-glutamine, 10 mmol/L HEPES, and 1% nonessential amino acids at 2.5×10^6 cells per 2 mL per well in 6-well tissue culture plates (Falcon; BD Biosciences, Oakville, Ontario, Canada) in the presence of recombinant human (rh) GM-CSF (20 ng/mL) and rhIL-4 (20 ng/mL) at 37°C in a humidified 5% CO₂ incubator for 5 to 7 days. When indicated, IL-4 was replaced by 20 ng/mL IL-15. On day 3, fresh medium supplemented with these cytokines was added. On day 5, 95% of cells displayed high CD1a, low CD83, low CD14, and medium CD86 expression and were used as iDCs.

For the generation of mDCs, day 5 iDCs were recultured for 48 hours in the same cytokine cocktail with addition of LPS (200 ng/mL). In selected experiments, DC maturation was induced with rhTNF- α (20 ng/mL) alone or in combination with rhIL-1 β (10 ng/mL), rhIL-6 (1000 U/mL), or PGE₂ (1 μ mol/L), or with polyI:C (25 μ g/mL) or zymosan A (50 μ g/mL). mDCs displayed a high expression of CD1a, CD83, and CD86.

Flow-cytometry analysis of MoDCs

For flow-cytometry studies, cells were washed with PBS and fixed with 2% paraformaldehyde for 15 minutes at room temperature. For intracellular staining, fixed cells were permeabilized with 0.1% saponin for an additional 15 minutes at room temperature. Then permeabilized and nonpermeabilized cells were incubated for 15 minutes with human IgG, resuspended with PBS-2% BSA, and labeled for 30 minutes with relevant antibodies or with isotype control antibody. Cells were then washed with PBS and incubated for 30 minutes with FITC-conjugated goat antirabbit IgG or with FITC-conjugated goat antimouse IgG. Finally, cells were washed again and resuspended in PBS, and immunofluorescence analysis of 5000 cells was performed on a FACScan flow cytometer (BD Biosciences, San Jose, Calif).

RNA isolation and real-time RT-PCR

Total RNA from DCs was isolated by use of Tripure Reagent (Roche Diagnostics, Indianapolis, Ind). RNA was digested with DNase 1 (Amersham Pharmacia Biotech, Baie d Urfé, Quebec, Canada) to exclude genomic DNA contamination, and first-strand cDNA synthesis was performed on 1 μ g using a M-MLV Reverse Transcriptase (Bio/Can Scientific Inc, Mississauga, Ontario, Canada). Real-time RT-PCR was performed by means of Rotor Gene RG-3000 (Corbett Research, Mortlake, NSW, Australia) and the Platinum Quantitative PCR SuperMix-UDG (Invitrogen, Burlington, Ontario, Canada) according to the protocol provided by the manufacturer. After initial denaturation for 2 minutes at 95°C, thermal cycling was performed for 45 cycles with steps of 95°C for 30 seconds, 60°C for 30 seconds, and 72°C for 30 seconds, with the fluorescence read at the end of each cycle. The following oligonucleotide primer sets were obtained from Sigma (Oakville, Ontario, Canada): human CysLT1R: forward, 5'-CCTCAGCACCTATGCTTTGT-3' and reverse, 5'-ATTGTCTTGTGGGGGCTCAA-3' (amplifying a 249-bp fragment); human CysLT2R: forward, 5'-AGACTGCATAAAGCTTTGGTTATC-3' and reverse, 5'-ATACTCTTGTTCCTTTCTCAACC-3' (amplifying a 196-bp); and human glyceraldehyde phosphate dehydrogenase (GAPDH): forward, 5'-GATGACATCAAGAAGGTGGTGAA-3' and reverse, 5'-GTCTTACTCCTTGGAGGCCATGT-3' (amplifying a 246-bp).

To assess CysLT1 mRNA stability, iDCs were treated for 16 hours with LPS or PGE₂ + TNF- α , and their transcriptional activity was then interrupted with actinomycin D (10 μ g/mL). CysLT1 mRNA decay was measured at intervals thereafter, and CysLT1 mRNA half-life was determined.

Calcium mobilization assay

Cells (3×10^6 /mL dendritic cells or 5×10^6 /mL monocytes) in HBSS buffer, containing 10 mmol/L HEPES and 0.1% FBS, were loaded with a Dulbecco modified Eagle medium solution of Fura 2/AM (1 mmol/L) at a final concentration of 3 μ mol/L for 45 minutes at room temperature. Before the calcium assay, cells were centrifuged and resuspended in the HBSS buffer with 2 mmol/L Ca²⁺. Changes in fluorescence were recorded by using a spectrofluorimeter. $[Ca^{2+}]_i = K_D([F - F_{min}]/[F_{max} - F])$ after calibration with Triton X-100 and EGTA. Cells were stimulated with LTD₄, LTC₄ or LTB₄ at 100 nmol/L or with platelet-activating factor (PAF) at 1 nmol/L final concentration. When indicated, cells were pretreated with the CysLT1 antagonist montelukast (10 μ mol/L) for 10 minutes before stimulation.

Chemotaxis assay

Chemotactic activity was performed with Boyden chambers by using a modified Boyden chamber chemotaxis assay. DCs were washed and resuspended in chemotaxis medium containing RPMI supplemented with 2 mmol/L L-glutamine, 10 mmol/L HEPES, and 0.25% lipid-free BSA. Assays were performed in duplicate by using 200,000 cells/200 μ L in the upper chamber. In selected experiments, iDCs or mDCs were pretreated for 30 minutes with antagonists of cysLTs. The lower chamber contained 200 μ L of the chemoattractants MIP-3 β (CCL19), LTD₄, LTC₄, or their vehicle diluted in chemotaxis medium. The 2 chambers were separated by a 5- μ m pore size polycarbonate filter (Osmonics, Westborough, Mass). Basal migration (negative control) was measured in the absence of chemoattractant (medium alone), and chemokinesis was measured in the presence of chemoattractants in both chambers. After incubation for 2 hours at 37°C in 5% CO₂, the chambers were disassembled, and the upper side of the filter was scraped free of cells. Cells on the lower side of the filter were removed with 5 mmol/L EDTA then combined with the cells migrated into the lower chamber. Finally, migrated cells were centrifuged, resuspended in 150 μ L PBS, and counted by flow

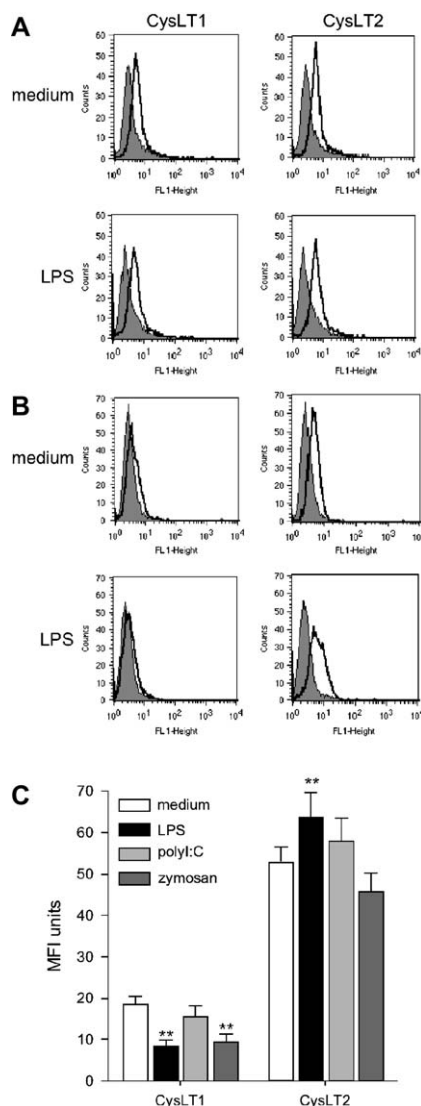


FIG 1. Expression of the leukotriene receptors CysLT1 and CysLT2 by monocytes (A) and MoDCs (B) exposed to medium or LPS (200 ng/mL) for 48 hours. C, Differential expression of CysLT receptors by MoDCs exposed to medium, LPS (200 ng/mL), polyI:C (25 μ g/mL), or zymosan (50 μ g/mL) for 48 hours. Shaded histograms (A and B) represent isotype control labeling, and open lines represent cognate antibody labeling. One representative experiment of 10 to 12 separate experiments is shown. Data in C are presented as mean fluorescence intensity (MFI) $\pm$ SEM; n = 5; **P < .005.

cytometry, acquiring events for a fixed period with the use of CellQuest software (BD Bioscience).

Statistical analysis

Statistical analysis was performed by using the Student *t* test or ANOVA, as appropriate. Significance was considered at *P* < .05.

RESULTS

Expression of leukotriene receptors on MoDCs

In a first series of experiments, we analyzed MoDC expression of CysLT1, CysLT2, and the LTB₄ receptor

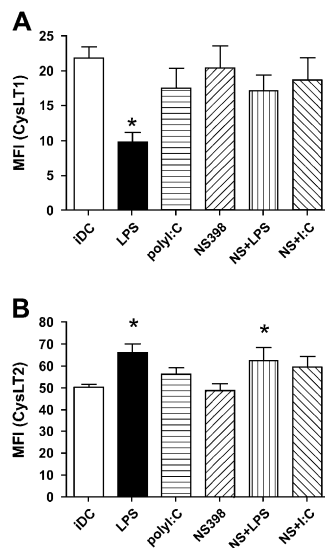


FIG 2. Effect of the COX-2 inhibitor NS398 (10 μ mol/L) on the modulation of CysLT1 (**A**) and CysLT2 (**B**) expression in DCs induced to mature with LPS (200 ng/mL) or polyI:C (25 μ g/mL) for 48 hours. Data are presented as mean fluorescence intensity (MFI) $\pm$ SEM; $n = 7$; * $P < .02$.

1 in relation to their differentiation and maturation state. In these experiments, differentiation of monocytes into MoDCs was induced by culture with GM-CSF + IL-4 and maturation was induced by a 48-hour exposure of immature MoDCs to LPS. Fig 1 illustrates the expression of leukotriene receptors by iDCs and mDCs (Fig 1, B) in comparison with that of medium-treated or LPS-treated monocytes (Fig 1, A). Monocytes readily expressed CysLT1 and CysLT2 receptors, as previously described,¹⁸ and the expression of these receptors was not altered by LPS treatment. In contrast, differentiation of MoDCs resulted in a relative downregulation of both receptors, most evident in terms of CysLT1 expression. Moreover, maturation of MoDCs with LPS resulted in an almost total loss of CysLT1 receptor expression, whereas CysLT2 expression was upregulated. Downregulation of CysLT1 and upregulation of CysLT2 receptor expression in MoDCs by LPS was rapid, evident at as early as 6 hours of treatment (data not shown).

Because LPS signals through interaction with Toll-like receptor (TLR)-4, we tested 2 other TLR agonists for their capacity to induce phenotypic changes in MoDCs. Downregulation of expression of CysLT1 was observed not only with LPS but also with zymosan (Fig 1, C), whereas exposure of MoDCs to polyI:C had no effect on CysLT receptor expression. Downregulation of CysLT1 expression was similar whether LPS was purified from *E coli*, *S minnesota*, or *S marcescens* (data not shown). Downregulation of CysLT1 by LPS could not be reproduced with the inflammatory cytokines TNF- α $\pm$ IL-1 β $\pm$ IL-6 (data not shown).

Because LPS is a known inducer of COX-2 activity and subsequent prostaglandin production, we investigated whether its effect on CysLT1 expression could be a result

of prostaglandin production. Fig 2, A, illustrates that the selective COX-2 inhibitor NS-398 reversed the downregulation of CysLT1 expression in LPS-matured DCs, whereas it did not affect polyI:C-matured DC. On the other hand, NS-398 had no effect on CysLT2 expression (Fig 2, B). PGE₂ or TNF alone had no significant effect on CysLT1 or CysLT2 expression, but when PGE₂ and TNF α were combined to induce DC maturation, they synergized in reducing CysLT1 expression to levels equivalent or lower than those induced by LPS (Fig 3, A). The addition of the phosphodiesterase inhibitor pentoxifyline further reduced CysLT1 expression, suggesting a role for cyclic adenosine monophosphate in the regulation of CysLT1 expression in DCs. In contrast, PGE₂, TNF- α , or their combination could not reproduce the upregulation of CysLT2 expression observed with LPS (Fig 3, B). The combination of TNF- α and PGE₂ constituted an effective maturational stimulus for human MoDCs in terms of CD86 expression, whereas PGE₂ alone was a poor stimulus, and TNF- α alone was only partially effective (data not shown).

Modulation of CysLT1 and CysLT2 expression could also be observed at the mRNA level, using real-time PCR (Fig 3, C and D). Maturation of DC with LPS reduced CysLT1 mRNA levels by 70%, whereas polyI:C had no effect. PGE₂ or TNF- α alone also had no effect, whereas their combination reduced CysLT1 mRNA levels by 80%. As with CysLT2 protein expression, CysLT2 mRNA levels were upregulated by LPS, but not by PGE₂ or TNF- α alone. Their combination, however, was inhibitory.

To assess whether treatment with LPS or PGE₂ + TNF- α affected CysLT1 mRNA stability, iDCs were treated for 16 hours with LPS or PGE₂ + TNF- α , and their transcriptional activity was then interrupted with actinomycin D. CysLT1 mRNA decay was measured at intervals thereafter. CysLT1 mRNA half-life was determined to be approximately 8 hours in untreated iDCs, whereas it was reduced to 4 hours by LPS and to less than 2 hours by PGE₂ + TNF- α .

Because differentiation of monocytes into MoDCs with IL-4 is associated with a downregulation of cytosolic phospholipase A₂ and COX-2 expression^{19,20} as well as 5-lipoxygenase,²¹ we replaced in selected experiments IL-4 + GM-CSF with IL-15 + GM-CSF to induce MoDCs.²² LT receptor expression in the latter cells was not altered, and downregulation of CysLT1 by LPS or PGE₂ + TNF- α was identical (data not shown).

In contrast with MoDCs, human monocytes were totally resistant to downregulation of CysLT1 expression by LPS (Fig 1, A) or PGE₂ + TNF- α (data not shown), indicating that the differentiation process leading to the DC phenotype was needed to render them sensitive to these biological modifiers.

Effects of cysLT on DC calcium mobilization

Human monocyte-derived iDCs responded with a robust calcium flux to stimulation with LTD₄ (Fig 4, A), albeit less by approximately 25% than monocytes

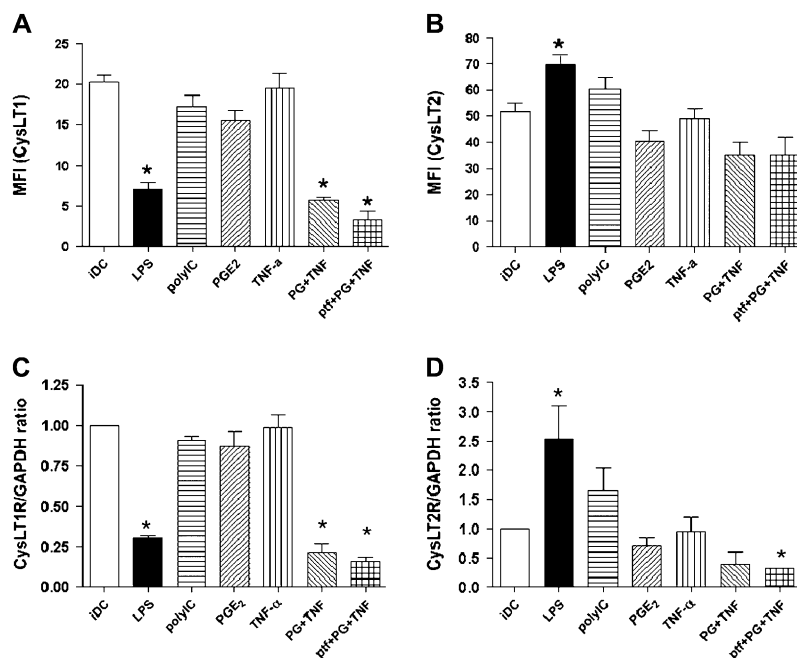


FIG 3. Modulation of CysLT1 (**A**) and CysLT2 (**B**) expression in DCs induced to mature with LPS (200 ng/mL), polyI:C (25 μ g/mL), PGE₂ (1 μ mol/L), TNF- α (20 ng/mL), PGE₂ + TNF- α , or PGE₂ + TNF- α + pentoxifylline (ptf; 1 mmol/L) for 48 hours. Data are presented as mean fluorescence intensity (MFI) $\pm$ SEM; $n = 7$; * $P < .02$. Modulation of CysLT1 (**C**) and CysLT2 (**D**) mRNA expression in DCs induced to mature with these stimuli for 48 hours. Real-time PCR analysis was performed and data are presented as CysLT/GAPDH ratios $\pm$ SEM; $n = 5$. * $P < .005$.

(Fig 4, G). The calcium flux was effectively prevented by pretreatment of the DCs with the CysLT1 antagonist montelukast (Fig 4, B). DCs induced to mature with LPS, however, failed to respond to LTD₄ in terms of calcium flux (Fig 4, C), but responded well to PAF (1 nmol/L; Fig 4, E). Pretreatment of the cells with the COX-2 inhibitor NS-398 partially restored their responsiveness to LTD₄ (Fig 4, D). DCs matured with polyI:C maintained their responsiveness to LTD₄ (Fig 4, F). Monocyte responses to LTD₄ in terms of calcium flux were not affected by LPS (Fig 4, G and H).

Effects of cysLT on DC migration

Immature DCs showed a significant chemotactic migration in response to LTD₄ (Fig 5). No chemokinetic activity was detected (data not shown). In contrast, LPS-treated, mDCs showed a comparatively much weaker chemotactic response to LTD₄, whereas polyI:C-treated, mDCs showed a responsiveness similar to iDCs (Fig 5).

Migration of iDCs to LTD₄ was mostly prevented by pretreatment with the CysLT1 receptor antagonists montelukast (1 μ mol/L) or LY171863 (5 μ mol/L) as well as with the dual CysLT1/CysLT2 antagonist and partial CysLT2 agonist BAYu9773 (2 mmol/L; data not shown). The weaker migration of LPS-treated mDCs in response to LTD₄ was also prevented by the antagonists, as was the stronger response of polyI:C-treated mDCs.

When graded concentrations of CCL19 were used in the presence of LTD₄, no significant additive effect was

observed with LPS-matured DCs (Fig 6, A). In contrast, polyI:C-matured DCs responded to LTD₄ with a significantly enhanced DC migration to CCL19 at 10 and 50 ng/mL of the latter (Fig 6, B).

DISCUSSION

We have shown that human MoDCs differentially express leukotriene receptors and respond to LTD₄ with cytosolic calcium flux and chemotactic activity. Whereas human monocytes express approximately equal levels of the leukotriene receptors CysLT1, CysLT2, and BLT1, their differentiation into immature MoDCs markedly reduces their expression of CysLT1. We have previously shown that IL-4 and IL-13 are potent enhancers of CysLT1 expression in monocytes and monocyte-derived macrophages within 24 hours.¹⁸ Our current data show that monocytes cultured for a prolonged period (5-7 days) with IL-4 and GM-CSF, to differentiate into DCs, down-regulate their expression of CysLT1. Differentiation of DCs using IL-15 + GM-CSF, which induces a Langerhans-like cell phenotype²² instead of IL-4 + GM-CSF, is equally effective in terms of differentiation and CysLT1 downregulation.

Maturation of MoDCs in response to LPS, but not to polyI:C, further reduces CysLT1, whereas it augments CysLT2 receptor expression. Monocyte expression of CysLT1 and CysLT2 is not affected, however, by LPS

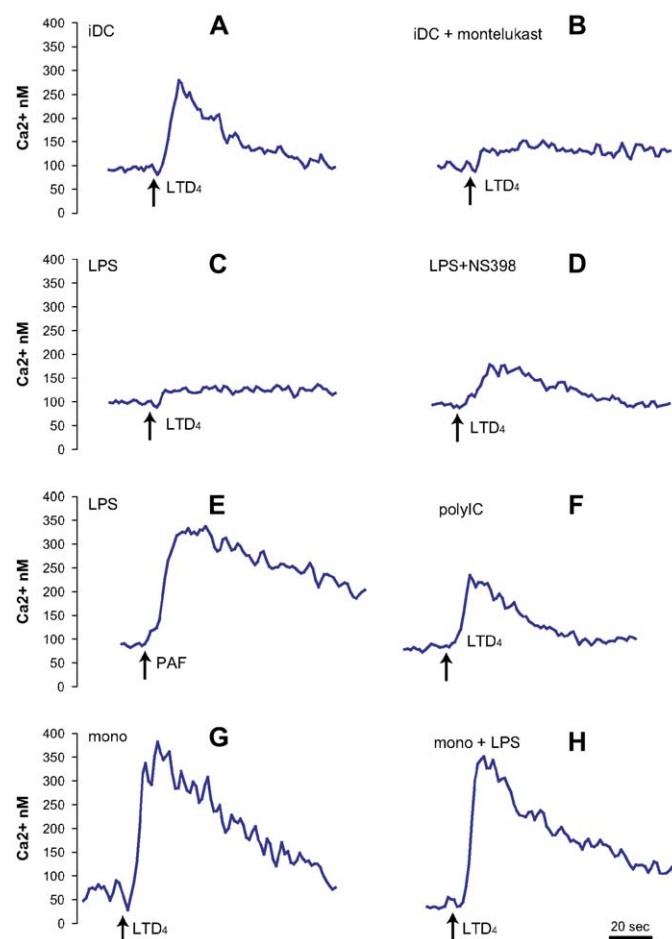


FIG 4. Cytosolic calcium mobilization in response to LTD₄ (100 nmol/L) stimulation was measured in iDCs in the absence (A) or presence (B) of montelukast (10 μ mol/L), as well as in DCs matured with either LPS (200 ng/mL) in the absence (C) or presence (D) of NS-398 (10 μ mol/L), or polyI:C (25 μ g/mL) (F) and in monocytes (mono) in the absence (G) or presence (H) of a 24-hour stimulation with LPS (200 ng/mL). As control, calcium mobilization in response to PAF (1 nmol/L) stimulation was measured in DCs matured with LPS (200 ng/mL) (E). Data are representative of 3 separate experiments.

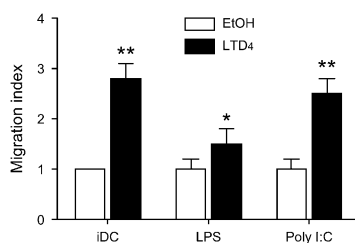


FIG 5. Chemotactic responses of DCs to LTD₄. iDCs or DCs induced to mature with LPS (200 ng/mL) or polyI:C (25 μ g/mL) were stimulated to migrate in response to LTD₄ (100 nmol/L). Spontaneous migration in the presence of vehicle alone was normalized to 1. $n = 4$; * $P < .05$; ** $P < .005$. EtOH, Ethanol.

treatment, suggesting that this effect is differentiation-dependent. Downregulation of CysLT1 expression by LPS is also evident at the mRNA level, and our data indicate that LPS reduces CysLT1 mRNA stability. Moreover, the LPS effect cannot be reproduced by inflammatory

cytokines, but is prevented by inhibition of COX-2 and reproduced by the combined action of TNF- α and PGE₂.

Downregulation of CysLT1 expression by LPS also has direct consequences on DC responsiveness to leukotriene. Cytosolic calcium flux in response to LTD₄ is significantly reduced in LPS-matured DCs compared with iDCs or polyI:C-matured DCs. Similarly, chemotactic responses of LPS-matured DCs to LTD₄ are impaired in comparison with those of iDCs or polyI:C-matured DCs.

The potential importance of the current observations is 2-fold. First, CysLT receptors may contribute to attracting iDCs to areas of allergic inflammation, such as the respiratory or digestive mucosa and the skin, in response to leukotrienes produced locally by resident (mast cells, macrophages, other DCs) or infiltrated (eosinophils) cells. During subsequent maturation, DCs would respond less to the chemotactic effect of leukotrienes, but leukotrienes would enhance DC responses to CCL19 via their newly acquired CCR7 receptor. Recently, Machida et al¹²

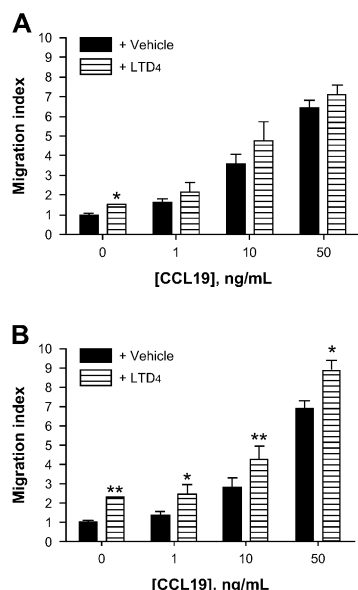


FIG 6. Chemotactic responses of DCs to graded concentrations of CCL19 in the presence or absence of LTD₄ (100 nmol/L) were assessed as described in Methods. Responses of MoDCs induced to mature with LPS (A) or polyI:C (B) to the chemoattractant are compared. n = 4; *P < .05; **P < .005.

reported that cysLTs increased IL-10 production by mouse myeloid DCs pulsed with the protease allergen *Dermatophagoides farinae* and enhanced their capacity to induce lung eosinophilia and IL-5 production in a murine asthma model. Using the CysLT1 antagonist pranlukast, Okunishi et al²³ also suggested a role for cysLTs in DC activation and antigen-induced immune responses in the lung in a mouse model of asthma.

Second, our observation that some (LPS) but not other (polyI:C) maturational stimuli downregulate CysLT1 and upregulate CysLT2 is intriguing both mechanistically and physiologically. It suggests that certain patterns of recognition of microbial products, and purportedly the involvement of different TLRs,²⁴ significantly and differentially affect leukotriene receptor expression and function. Physiologically, this may translate into the capacity of certain bacterial (LPS) or fungal (zymosan) products to inhibit DC responsiveness to leukotrienes, whereas certain viral (polyI:C) stimuli would not.

Toll-like receptor 4 is responsible for enabling responses to LPS,²⁵ as well as to a wide variety of exogenous and endogenous molecules (see review²⁶). TLR2, which needs to heterodimerize with either TLR1 or TLR6, enables responses to a range of molecules including zymosan, peptidoglycans, and lipopeptides.²⁷ TLR3 was shown to recognize double-stranded RNA, including polyI:C.²⁸ Signaling through TLRs involves both shared and TLR-specific pathways. A shared pathway mediated through the Toll-IL-1R signaling domains results in activation of nuclear factor- κ B and the mitogen-activated protein kinase pathways (c-Jun N-terminal kinase, p38, extracellular signal-regulated kinase 1/2).²⁹⁻³³ On the other hand, selective TLR signaling pathways include

the MyD88 adaptor protein-independent activation of IFN regulatory factor 3, which has been implicated in generation of type I IFNs. TLR3, TLR9, and TLR4 but not TLR2 activation results in the production of IFN- β .³⁴ Moreover, activation of DCs by TLR4 or TLR2 agonists, although it led to comparable activation of nuclear factor- κ B and mitogen-activated protein kinase, resulted in strikingly different patterns of cytokine and chemokine gene transcription.³⁵ TLR4 activation promoted the production of IL-12 p70 and the chemokine interferon-induced protein (IP)-10, which are associated with T_H1 responses, whereas activation of TLR2 failed to induce IL-12 p70 or IP-10 but resulted in the release of antagonistic IL-12 p40 homodimers, which would favor T_H2 development, as well as IL-8 and IL-23 p19.

In conclusion, our data suggest that selective TLR agonists can modulate MoDC expression of CysLT receptors and cysLT-mediated functions. Conversely, leukotrienes can modulate DC functions, namely cytosolic calcium mobilization and chemotaxis, and potentially affect both innate and adaptive immunity in the context of allergic inflammation. The nature and extent of this modulation may be primarily dependent on the maturational environment of DCs. Ongoing studies in our laboratory are aimed at defining the effect that our current findings may have on antigen presentation and processing by DCs, and on their modulation of T-cell responses, especially as they relate to human allergic airway disease.

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