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Cysteiny1-Leukotriene Receptor Type 1 Expression and Function Is Down-Regulated during Monocyte-Derived Dendritic Cell Maturation with Zymosan: Involvement of IL-10 and Prostaglandins

Maryse Thivierge, Jana Stankova, and Marek Rola-Pleszczynski

TLRs sense microbial products and initiate adaptive immune responses by activating dendritic cells (DCs). DCs have been shown to produce leukotrienes and, conversely, leukotrienes are known to modulate several DC functions. In this study, we examined the modulation of expression and function of cysteiny1-leukotriene receptor type 1 (CysLT1) on human monocyte-derived DCs during their differentiation and subsequent maturation with zymosan, a TLR2 agonist. Maturation of DCs with zymosan reduced CysLT1 mRNA levels and protein expression in a time-dependent fashion and was associated with a diminution of functional responsiveness to leukotriene D4, as assessed by intracellular calcium mobilization, CCL2 and CCL3 production, and chemotaxis. The effect of zymosan was mediated by both TLR2 and dectin-1 activation. Zymosan also induced a rapid expression of cyclooxygenase-2 and the production of PGE2 and IL-10. Addition of an anti-IL-10 neutralizing Ab or inhibitors of cyclooxygenase greatly reduced the ability of zymosan to down-regulate CysLT1 expression. Down-regulation of CysLT1 expression by zymosan could be reproduced by a combination of IL-10 and PGE2, and was dependent on MAPK activation. Taken together, our findings indicate that zymosan down-regulates CysLT1 expression.

Dendritic cells (DCs) are considered to be the key APCs for the activation of naive T cells and for the initiation of the primary immune reaction (1–3). DCs exist in two functionally and phenotypically distinct states termed immature and mature. Immature DCs (iDCs) efficiently capture Ag but only express modest levels of MHC class II and costimulatory (CD40, CD80, CD86) molecules at the cell surface. Following maturation, however, DCs express high levels of MHC class II and costimulatory molecules, but capture Ag poorly (4, 5). DCs are, at this stage, best equipped to present Ag, which was taken up and processed during their immature stage, to T cells because they interact in secondary lymph nodes.

It is now clear that DCs can recognize microbial components using various pattern recognition receptors, such as the family of TLRs. Different TLRs expressed on DCs can discriminate between distinct pathogen-associated molecular patterns and initiate signaling pathways to induce DC maturation and activation (7, 8). The specific pathogen-associated molecular patterns recognized by TLR family members have been well characterized (9–15). Stimulation of TLRs by these ligands triggers several intracellular signaling pathways (8) and results in induction of antimicrobial activity (16) and the production of several cytokines including IL-1β, IL-6, IL-8, IL-10, IL-12, and TNF-α (2, 7, 17, 18).

Cysteiny1-leukotriene (cysLT), leukotriene (LT)C4, LTD4, and LTE4 are lipid mediators of inflammation, which play important roles in several chronic inflammatory diseases, including asthma and allergic rhinitis. They are produced by eosinophils, mast cells, basophils, monocytes, macrophages, and DCs from arachidonic acid through the 5-lipoxygenase pathway (19). The biological action of cysLT is primarily mediated via two known G protein-coupled receptors designated cysLT receptor type 1 (CysLT1) and cysLT receptor type 2 (CysLT2) (20–22). CysLT2 has higher affinity for LTD4 than for LTC4 and is expressed mainly in bronchial smooth muscle and in most leukocyte populations (23). CysLT2 has equal affinity for LTC4 and LTD4, and is widely expressed in many tissues, including brain, adrenal, lung, spleen, endothelium, and leukocyte (20).

Recent data support the hypothesis that, besides their well-established role in inflammation, cysLT might also have an important regulatory effect in leukocytes such as DCs. It has been shown that LTC4 promotes chemotaxis to CCL19 and mobilization of DCs from the skin to lymph nodes (24). Recently, cysLTs were shown to increase IL-10 production by allergen-pulsed mouse myeloid DCs and enhance their capacity to induce lung eosinophilia and IL-5 production in a murine asthma model (25). Other investigators reported the expression of 5-lipoxygenase in human Langherans cells and in human DCs generated from CD34+ hematopoietic progenitors (26, 27). It has been shown that splenic DCs,
isolated from mice treated with a CysLT1 antagonist, release a lower quantity of both IL-10 and IL-12 p70 upon ex vivo stimulation with LPS (28). In addition, pretreatment of patients with mild asthma using a CysLT1 antagonist decreased the number of circulating myeloid DCs, demonstrating a significant role of cysteine LTs in DC trafficking in vivo (29). Finally, a recent study showed that LTs can modulate cytokine release from DCs (30). Despite these observations, LT receptor expression in DC has not been extensively examined. Recently, we reported that LPS, a classic TLR4 agonist, down-regulates CysLT1 expression and cysLT-mediated function in human monocyte-derived DCs (MoDCs) via production of TNF-α and PGs, whereas the TLR3 agonist polyinosinic-polycytidylic acid (poly(I:C)) does not affect CysLT1 expression (31).

In the present study, we investigated the effects of zymosan, a TLR2 agonist, on CysLT1 modulation in MoDCs. Our data suggest that zymosan can inhibit CysLT1 expression, an effect mediated by the endogenous production of IL-10 and PGs, which are rapidly induced by TLR2 stimulation. These effects are dependent on TLR2 and dectin-1 activation by zymosan.

Materials and Methods

Reagents

Human recombinant GM-CSF, IL-4, IL-10, and CCL19 (MIP-3β) and a rabbit polyclonal anti-human IL-10 Ab were purchased from PeproTech. Platelet-activating factor (PAF), LTD4, PGD2, cyclooxygenases (COX) inhibitors indomethacin and NS-398 were obtained from Cayman Chemical. The CysLT1 selective antagonist montelukast was a gift from Merck-Frosst. Functional grade purified anti-human TLR2 (TL2.1) was purchased from eBioscience. Rabbit polyclonal anti-human CysLT1 Ab was obtained from Cayman Chemical; rabbit IgG isotype control was obtained from (BD Biosciences); FITC-conjugated goat anti-mouse IgG and FITC-conjugated goat anti-rabbit IgG and FITC-conjugated goat anti-mouse IgG were obtained from Bio/Can Scientific. From Frosst. Functional grade purified anti-human TLR2 (TL2.1) was purchased from (BD Biosciences); FITC-conjugated goat anti-rabbit IgG and FITC-conjugated goat anti-mouse IgG. Finally, cells were washed with FITC-conjugated goat anti-rabbit IgG or with isotype control Ab. After incubation, cells were washed with PBS and incubated for 30 min with FITC-conjugated goat anti-mouse IgG. Intracellular (C terminus) domain of the receptor. Permeabilized cells were incubated with 0.1% saponin for an additional 15 min at room temperature. This step was necessary because the anti-CysLT1 Ab is directed toward an intracellular (C terminus) domain of the receptor. Permeabilized cells were then incubated for 15 min with human IgG to block binding to Fc receptors, resuspended with PBS-2% BSA, and labeled for 30 min with relevant Abs or with secondary Abs. After incubation, cells were washed with PBS and incubated for 30 min with FITC-conjugated goat anti-rabbit IgG or with FITC-conjugated goat anti-mouse 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) supported by CellQuest software (BD Biosciences).

RNA isolation and RT-PCR

RNA was purified by using TRIzol reagent (Invitrogen) according to the manufacturer’s instructions. After treatment with DNase I to exclude genomic DNA contamination, 1.0 μg of RNA was converted to cDNA with oligo(dT) (Amersham Biosciences) and reverse transcriptase (M-MLV; Promega) in a volume of 20 μL. PCR was performed in a final volume of 50 μL containing reaction buffer, dNTP, forward and reverse oligonucleotide primers, TaqDNA polymerase (New England Biolabs), and 2 μl of reverse transcriptase reaction product. RT-PCR was performed by means of a Biometra thermocycler (Montreal Biotech). PCR was done under the following conditions: 1 cycle at 94°C for 1 min, followed by 34 cycles (for CysLT1, IL-10, and GAPDH) or 24 cycles (for GAPDH) containing 30 s denaturation at 94°C, 30 s annealing at 60°C, and 40 s extension at 72°C, followed by a final 8 min extension at 72°C. Controls in which the reverse transcription step was omitted confirmed that the PCR products reflected mRNA levels rather than contaminating genomic DNA. PCR products (10 μl) were electrophoresed in a 1.5% (w/v) agarose gel and visualized with ethidium bromide. Densitometric quantification was performed by using NIH Image J software (version 1.32). The following oligonucleotide primer sets were obtained from IDT: human CysLT1 (forward) 5′-CTCTGACACCTGCTGCTGTTG-3′ and (reverse) 5′-ATT GTCCGTGAGGCCGCTAACA-3′; human IL-10 (forward) 5′-ACAGCTC AGCAGCTCAGCTGTT-3′ and (reverse) 5′-AGTTTCATGTGCCGCTTGTA-3′; human COX-2 (forward) 5′-ATGAGTGGTGGGAAATTACGT-3′ and reverse, 5′-GATCATCTGCTGCTGATGC-3′; and human GAPDH (forward) 5′-GATGACATCAAGAGGGTGGTGA-3′ and (reverse) 5′-GTCTTACTCTGGCGGAGCATG-3′.

Real-time quantitative PCR

In selected experiments, DCs were stimulated with 10 nM LTF4, for 4 h. Total RNA was purified by using TRIzol reagent (Invitrogen) according to the manufacturer’s instructions. The 1.0 μg of RNA was converted to cDNA with oligo(dT) and reverse transcriptase (M-MLV) from Promega in a volume of 20 μL. CCL2, CCL3, and GAPDH expression were measured using real-time PCR performed on a Rotor-Gene 3000 (Corbett Research). The following oligonucleotide primer sets were used: human CCL2 (forward) 5′-CATAGACAGGAGCCTAGGCT-3′ and (reverse) 5′-GATTGAGCATGACAAAGGTTGCTGCAG-3′ and (reverse) 5′-GTCTTACTCTGGCGGAGCATG-3′. Each sample for the real-time PCR consisted of 1 μl of cDNA, 2.5 mM MgCl2, 100 μM dNTP, 1 μM primer, 2.5 μl of 10X PCR buffer, 0.5 U of Taq polymerase (New England Biolabs) and 0.8 μl of SYBR Green (1/1000 stock dilution; Molecular Probes) in a reaction volume of 25 μl. The cycling program consisted of an initial denaturation at 95°C for 5 min, at 40 cycles of amplification at conditions 95°C for 20 s, 58°C for 20 s, and a final extension at 72°C for 6 min. Comparison of the expression of each gene between its control and stimulated states was determined with the comparative threshold value (ΔΔCt) method, according to the formula (ΔCt (Ct G.O.I-Ct Ci - Ct HK.G-Ct Ci) - ΔCt (Ct G.O.I-Ct Si - Ct HK.G-Ct Si)). Results were then transformed into fold variation measurement as fold increase of 2^ΔΔCt.

IL-10 and PGE2 assays

IL-10 was measured in DC supernatants by means of an OptEIA kit from BD Pharmingen. PGE2 production was determined with an EIA kit from Cayman Chemical.

Calcium mobilization assay

DCs (3 × 10^6/ml) in HBSS buffer, containing 10 mMol/L, HEPES and 0.1% BSA, were loaded with fura 2-AM (Molecular Probes) at a final concentration of 3 μMol/L for 30 min at room temperature. Before the calcium assay, cells were washed and resuspended in HBSS buffer containing 2 mMol/L Ca^2+. Cells were stimulated with vehicle, LTD4 at 100 mMol/L or with PAF at 10 mMol/L final concentration. Analysis was performed using a spectrophotometer (Hitachi F-2000) and an intracellular calcium fluorescence solutions software (version 1.0). When indicated, cells were pretreated with the CysLT1 antagonist montelukast (10 μMol/L) before stimulation with LTD4.
Biosciences). The lower chamber contained 200,000 cells that were migrated into the lower chamber. Finally, migrated cells were centrifuged, resuspended in 150 μl of PBS, and counted by flow cytometry, expressed as geometric mean fluorescence intensity (MFI). The iDCs were incubated with or without zymosan (25 μg/ml) and their transcriptional activity was then interrupted with actinomycin D (10 μg/ml). Data were normalized to time t = 0 and represent mean ± SEM for n = 4 experiments. *, p < 0.05.

Chemotaxis assay

Chemotactic activity was performed with Boyden chambers using a modified Boyden chamber chemotaxis assay. DCs were washed and resuspended in chemotaxis medium containing RPMI 1640 and 0.25% BSA. Assays were performed in duplicate using 200,000 cells/200 μl in the upper chamber. The lower chamber contained 200 μl of the chemotacticants CCL19 (MIP-3β), LTD₄, or the vehicle diluted in chemotaxis medium. The two chambers were separated by a 5-μm diameter polycarbonate filter (Osmonics). Basal migration (negative control) was measured at different time intervals. CysLT1 mRNA decay was measured by flow cytometry, expressed as geometric mean fluorescence intensity. Data represent mean ± SEM for n = 5 experiments. *, p < 0.05.

Statistical analysis

Statistical analysis was performed using Student’s t test or ANOVA with Bonferroni post hoc test, as appropriate. Differences with a value for p < 0.05 were considered significant.

Results

Zymosan reduces CysLT1 receptor expression on MoDC

Fig. 1, A and C, illustrates CysLT1 mRNA and protein expression, respectively, in iDCs incubated with zymosan (25 μg/ml) for different periods of time. Kinetics show a significant 30% reduction in CysLT1 mRNA levels within 8 h of treatment with zymosan, with a maximum reduction of 75% obtained after 24 h of incubation. The effect was concentration-dependent, being evident at 5 μg/ml with a plateau at 25–50 μg/ml zymosan treatment (Fig. 1B). Analysis by flow cytometry revealed a stable level of CysLT1 protein expression in untreated iDCs (Fig. 1C), without consideration of time. However, a significant decrease in CysLT1 expression was detectable after 12 h of treatment with zymosan. CysLT1 levels continued to decrease until 48 h of stimulation, with a greater than 65% reduction of total protein expression. These results indicate that exposure to zymosan led to CysLT1 down-regulation in iDCs in terms of both mRNA and protein expression. To assess whether treatment with zymosan influenced CysLT1 mRNA stability, iDCs were incubated with zymosan and their transcriptional activity was then interrupted with actinomycin D (Fig. 1D). CysLT1 mRNA decay was measured at different time intervals. CysLT1 mRNA half-life was determined to be ~8–10 h in iDCs, whereas zymosan reduced it to 6–7 h, suggesting that at least part of CysLT1 mRNA down-regulation by zymosan occurred at a posttranscriptional level. We had previously shown that LPS induced a down-regulation of CysLT1 expression in MoDCs, but not in monocytes (31). In contrast, zymosan was able to down-regulate CysLT1 expression in both DCs and monocytes (Fig. 1E).

We then investigated the receptors through which zymosan particle-mediated their effects. It was previously shown that TLR2 is required for zymosan-induced production of cytokines by macrophages (32). A variety of receptors, including those for mannans and β-glucans, have also been implicated in recognition and phagocytosis of zymosan particles (33). Recently, Brown et al. (34) identified a C-type lectin called dectin-1 that is expressed on monocytes, macrophages, and DCs as a phagocytic receptor for zymosan. However, in the present study, we found that zymosan treatment did not induce the expression of dectin-1 in either iDCs or MoDCs (Fig. 2A).

The expression of dexamethasone-inducible receptor-1 (DIR-1) was also examined. DIR-1 is a receptor that is induced by dexamethasone treatment and is known to bind to zymosan particles (35). While DIR-1 expression was undetectable in iDCs, it was induced by treatment with zymosan in MoDCs (Fig. 2B). These findings suggest that zymosan may be recognized by DIR-1 in MoDCs, but not in iDCs, and that this recognition is not required for the down-regulation of CysLT1 expression by zymosan.
attachment and ingestion of zymosan particles. Laminarin (a soluble β-glucan from the brown seaweed L. digitata) can block this recognition. Moreover, an additional β-glucan-independent mechanism could be inhibited by mannan. As illustrated in Fig. 2, zymosan-reduced CysLT1 expression by human MoDCs was significantly prevented by laminarin, but not by mannan. DC pretreatment with anti-TLR2 Ab also partially blocked the effect of zymosan on CysLT1 expression. Treatment of zymosan with hot alkali was shown to remove all of its TLR-stimulating properties and to separate the dectin-1- and TLR2-activating components of zymosan particles (35): thus, alkali-treated zymosan activates dectin-1 without stimulating TLRs. In our system, we observed that sodium hydroxide-treated zymosan was less efficient in down-regulating CysLT1 expression and that its combination with laminarin restored CysLT1 expression close to iDC levels. Taken together, these data suggest an important role for both dectin-1 and TLR2 in the reduction of CysLT1 expression by zymosan in human DCs.

**Implication of IL-10 and PGs in down-regulation of CysLT1 expression**

The onset of CysLT1 down-regulation with zymosan was quite slow with a delayed reduction of transcript levels and protein expression that became only significant after several hours of stimulation. One possible mechanism through which TLR activation may reduce the expression of LT receptors would be through the release of an inhibitory factor. It is known that TLR activation that initiates DC maturation and function is accompanied by production of endogenous proinflammatory agents, such as TNF-α, IL-6, IL-12, and 5-lipoxygenase products such as LTB₄, as well as anti-inflammatory molecules, such as IL-10 and PGE₂ (2, 7, 17, 18 and reviewed in Ref. 36).

Supernatants were therefore collected from DCs treated for 24 h with zymosan and added to iDCs and poly(I:C)-treated DCs. These supernatants caused a diminution in DC expression of CysLT1, suggesting that secreted soluble factors alone were able to reduce CysLT1 expression (data not shown). We next tested the effect of zymosan directly on IL-10 and PGE₂ production by DCs. As shown in Fig. 3A, zymosan induced a rapid induction of COX-2 mRNA expression (Fig. 3A), whereas COX-1 mRNA expression was not affected (Fig. 3B). This effect was associated with a rapid production of PGE₂ in the supernatants detected as early as 1 h, maximal by 8–12 h and maintained until 24 h (Fig. 3C). In parallel experiments, zymosan induced a significant increase in IL-10 mRNA levels, beginning within 2 h with a maximum obtained between 8 and 12 h of stimulation (Fig. 3D). At 24 h, IL-10 mRNA levels had decreased, indicating that the effect of zymosan was rapid but transient. Fig. 3E shows an enhanced and time-dependent IL-10 protein accumulation observed in DC supernatants in response to zymosan stimulation that started at 4 h, was maximal by 12 h and was maintained through 24 h. Sodium hydroxide-treated zymosan was 50% less efficient in inducing IL-10 production and its combination with laminarin reduced it by 80% (data not shown).

CysLT1 expression on DCs was partially, but significantly inhibited by exogenous IL-10 or PGE₂ alone (Fig. 4A), whereas incubation with IL-10 in combination with PGE₂ markedly reduced CysLT1 expression to a level that was not statistically different from that induced by zymosan (Fig. 4B). Moreover, the combination of anti-IL-10 Ab and the COX inhibitor indomethacin during maturation of DCs with zymosan maintained 90% of CysLT1 expression compared with untreated iDCs. Our results suggest that the combination of both endogenous IL-10 and PGs, produced through zymosan stimulation of iDCs is able to downregulate CysLT1 expression.

Because PGs and TNF-α had been shown to play a major role in LPS-induced down-regulation of CysLT1 (31), and because both LPS and zymosan can induce TNF-α, IL-10, and PG production, we directly compared anti-TNF-α plus indomethacin and anti-IL-10 plus indomethacin treatment in both LPS- and zymosan-matured DCs in terms of CysLT1 expression. As shown in Fig. 4C, anti-TNF-α plus indomethacin did not reverse the inhibitory effect of zymosan, nor did anti-IL-10 plus indomethacin reverse the inhibitory effect of LPS.

**Implication of MAPK in modulation of CysLT1 expression by zymosan**

Signaling through TLRs results in activation of several MAPKs, including ERK1/2, stress-activated protein kinase JNK, and p38 MAPK (38, 39). Activation of p38 MAPK and ERK is involved in regulating cytokine expression in DCs (40, 41). Recently, zymosan was shown to regulate DC secretion of IL-10 via a mechanism dependent on activation of ERK (42). To determine whether MAPK signaling was responsible for CysLT1 down-regulation by zymosan, we used pharmacological reagents that inhibit MAPK pathways with different specificities: U0126 or PD98059, SB203580, and SP600125, selectively inhibiting MEK1/2, p38, and JNK, respectively. MoDCs were pretreated with each inhibitor for 30 min, followed by zymosan stimulation. As illustrated in Fig. 5A, U0126, PD98059 and SB203580, but not SP600125 treatment partially blocked the inhibitory effect of zymosan. A combination of U0126 or PD98059 with SB203580 essentially prevented the inhibitory effect of zymosan on CysLT1 expression. The roles of ERK and p38 pathways in the regulation of IL-10 production by zymosan were also investigated. Pretreatment with U0126 or the p38 inhibitor alone significantly reduced IL-10 production in MoDCs after zymosan stimulation, whereas simultaneous inhibition of both ERK and p38 activation brought down IL-10 production to near baseline levels (Fig. 5B). These results suggest that p38 and ERK MAPK pathways are an essential component of the mechanism for zymosan-induced down-regulation of CysLT1 expression and function.

**Zymosan modulates DC responsiveness to LTD₄**

We investigated whether the inhibition of CysLT1 expression in human DCs by zymosan was associated with a reduced biological...
responsiveness to LTD₄. The biological activity of the receptor on DCs was assessed by measuring intracellular calcium flux (Fig. 6), chemokine production (Fig. 7), and chemotaxis (Fig. 8) in response to LTD₄.

Stimulation of iDCs with LTD₄ induced a rapid calcium mobilization, an effect prevented by pretreatment with the CysLT₁ antagonist montelukast (Fig. 6). DCs matured with zymosan responded very weakly to LTD₄ when compared with iDC, whereas they were still able to respond to PAF stimulation. In concordance with our observations on CysLT₁ protein expression, the combination of laminarin and sodium hydroxide-treated zymosan induced no loss of DC responsiveness to LTD₄. DCs matured with poly(I:C) maintained their responsiveness to LTD₄.

Chemokine production by DCs in response to LTD₄ was also inhibited following maturation with zymosan (Fig. 7). LTD₄ induced the production of significant amounts of the chemokines CCL2 and CCL3 by iDCs. However, following maturation with zymosan, DCs lost their ability to produce either chemokine. This was partially reversed when anti-IL-10 Ab and indomethacin were added to zymosan during the maturation stage. In contrast to zymosan, poly(I:C)-induced DC maturation preserved CCL2 and CCL3 production in response to LTD₄. DC maturation as measured by CD83 and CD86 expression was equivalent in cells matured in the presence of poly(I:C), zymosan or zymosan plus anti-IL-10 with indomethacin (data not shown).

In chemotaxis assays, iDCs showed a significant chemotactic migration in response to LTD₄, whereas DCs incubated for 48 h with zymosan showed a total loss of chemotactic response to LTD₄ (Fig. 8A). As previously shown (31), maturation of DCs with poly(I:C) had no significant effect on CysLT₁ expression and was associated with a chemotactic responsiveness to LTD₄, albeit slightly weaker than that of iDCs. In parallel experiments, a partial restoration of responsiveness to LTD₄ in terms of migration was observed when endogenous IL-10 and PG production were blocked during maturation with zymosan. Incubation of DCs with IL-10 in combination with PGE₂ was as effective as zymosan in inhibiting DC migration to LTD₄.

However, not all chemotactic activity of DCs was decreased by zymosan. Recruitment of DCs into peripheral tissues and secondary lymphoid organs is driven by chemokine receptors expressed on the DC membrane (37): iDCs express CCR1, CCR5, and CCR6, whereas mDCs express CCR7, which renders them responsive to the chemokine CCL19 (MIP-3B). Maturation with zymosan or poly(I:C) was accompanied by an enhanced DC migration to CCL19, which was unaffected by anti-IL-10 Ab or indomethacin and which could not be reproduced by IL-10 and PGE₂ (Fig. 8B).
Discussion

DCs are believed to be critical in both initiating and modulating immune responses, playing a central role not only as immune sentinels in trafficking from the blood to primary inflammatory sites but also in their ability to capture, process, and transport Ags to secondary lymphoid tissues, to activate T cells. Several findings indicate that LTs play a central role in inflammatory conditions involving DCs, by activation of chemotaxis and other effector functions (24–26, 28–31, 43).

In the present study, our data suggest that the yeast cell wall, zymosan, previously considered to be a proinflammatory stimulus, is a potent down-regulator of CysLT1 expression in human DCs both in terms of protein and mRNA levels with a reduction in CysLT1 mRNA stability. Our data also suggest that TLR2, the major TLR activated by zymosan, is essential for zymosan-mediated inhibition of CysLT1 expression, but TLR2 is not the only receptor implicated. Of several zymosan receptors that are likely activated in our system, the β-glucan receptor dectin-1 is also essential, whereas the mannose receptor is not. The effect of zymosan on CysLT1 expression is reproduced by the combined action of IL-10 and PGE2 and can be partially prevented when endogenous IL-10 is neutralized and PG synthesis is prevented. These results suggest that the inhibitory action of zymosan is mediated by an IL-10- and PG-dependent mechanism. During preparation of the publication of this study, Woszczek et al. (44) also showed that IL-10 could down-regulate CysLT1 expression and function.

Down-regulation of CysLT1 expression by zymosan also affects DC responsiveness to cysLTs. Cytosolic calcium flux and chemotactic responses of zymosan-matured DCs to LTD4 are impaired in comparison to those of iDCs or poly(I:C)-matured DCs. This loss of responsiveness to cysLTs does not appear to be related to the maturation process of DCs, but rather to the specificity of the stimuli they encounter. Hence, as we reported previously (31) and in the present study, DCs matured with poly(I:C) maintain their levels of CysLT1 expression and responsiveness to LTD4 in terms of calcium mobilization and chemotaxis when compared with iDCs. Furthermore, cotreatment of DCs with IL-10 and PGE2 is accompanied by a markedly reduced expression of CysLT1 and chemotaxis to LTD4 without induction of DC maturation.
It is known that DC maturation induced by TLR activation is accompanied by production of large amounts of endogenous pro-inflammatory agents such as IL-1, TNF-α, IL-6, and IL-12 and anti-inflammatory molecules such as PGE₂ and IL-10. The anti-inflammatory effect of IL-10 is due, at least in part, to its potent biologic action on APC, in particular DCs. IL-10 strongly inhibits the activation of these cells, resulting in a reduced production of proinflammatory cytokines (45–47) and leads to diminished T cell stimulation (48). Both PGE₂ and IL-10 have similar immunosuppressive effects on APC. It was previously shown that PGE₂ has an inhibitory effect on DCs, reducing their maturation and their ability to present Ag (49). The effect of PGE₂ is prolonged and can be sustained by other factors, especially IL-10. In fact, several observations suggest that some inhibitory effects of PGs on DC functions are mediated via an IL-10-dependent mechanism (50, 51). In bone marrow-derived DC, COX-2-generated PGE₂ was shown to enhance the production of endogenous IL-10, which down-regulated IL-12 production and the APC function of bone marrow-derived DC (50). PGE₂ was shown to promote IL-10 production, which inhibited 5-lipoxygenase-activating protein expression and LTB₄ production from DCs (52) as well as their production of IL-6-, TNF-α, and COX-2-derived prostanoids (53). Autocrine production of IL-10 is a major determinant of the inhibited release of various proinflammatory mediators by APC, in particular DCs. Our observations that endogenous IL-10 might be an autocrine regulator of human DCs following zymosan stimulation correlate with previous work, which reported that IL-10 induction is an integral part of TLR signaling, likely influencing maturation and function of DCs and suggested that IL-10 acts in an autocrine manner to dampen DC activity (45–47). The mechanisms underlying the various actions of IL-10 are partially understood. The immunosuppressive and anti-inflammatory activities of IL-10 may be at least in part ascribed to its capacity to inhibit NF-κB function by blocking IκB kinase activity (54). The selective induction of nuclear translocation and DNA-binding of the suppressive p50/p50 homodimer is an important anti-inflammatory mechanism used by IL-10 to suppress inflammatory gene transcription (55). More recently, it was reported that IL-10 activates STAT3 to act indirectly by selectively inhibiting gene transcription, independently of the general effects on NF-κB, or by affecting posttranscriptional mRNA processing through an overall reduction in transcriptional rate of specific genes (56).

Signaling through TLRs involves both shared and TLR-specific pathways. A shared pathway mediated through the Toll/IL-1R (TIR) signaling domains results in activation of NF-κB and several MAPKs, including ERK1/2, stress-activated protein kinase JNK, and p38 MAPK (38, 39). According to our data, the signaling events triggered by zymosan that lead to down-regulation of CysLT1 involve p38 and ERK MAPK pathways. The same pathways appear to be involved in the modulation of both CysLT1 expression and IL-10 production by zymosan in our system. These observations are in agreement with recent results showing that, when macrophages and DCs are stimulated with LPS, TLR2 agonists or zymosan, at least three types of signaling pathways are activated: p38 MAPK and NF-κB activation (57), ERK activation (58), or Syk activation (59), respectively, all of which converge on the IL-10 gene and induce its expression. Moreover, activation of DCs by TLR4 or TLR2 agonists, although it led to comparable activation of NF-κB and MAPK, resulted in strikingly different patterns of cytokine and chemokine gene transcription. Some studies have noted that there is ligand selectivity in IL-10 induction;
for example, TLR2 ligands (peptidoglycan and zymosan) were reported to be more potent than TLR4 ligand (LPS) or TLR3 ligand (poly(I:C)) in inducing IL-10 (47, 48). Our results are in agreement with these reports, as we observed that zymosan and peptidoglycan were better inducers of IL-10 production when compared with LPS and poly(I:C) (data not shown) and that anti-IL-10 Ab was effective in preventing zymosan-mediated, but not in LPS-mediated down-regulation of CysLT1 expression. Interestingly, despite their use of several common signaling molecules, qualitative differences exist in the cellular responses elicited by ligands for different TLRs (38). It is believed that collateral signaling pathways may be responsible for this diversity.

Accumulating evidence indicates that the signaling specificity of each TLR is achieved through the selective use of TIR-containing adaptors (reviewed in Refs 38, 39). TLR3 and TLR4 use a MyD88-independent signaling pathway that involves the adaptor molecule TIR domain-containing adaptor-inducing IFN-β (TRIF). Further complexity in the TLR signaling pathways results from the obligatory use of the TIR adaptor protein TIRAP in association with MyD88 by TLR2 and TLR4, and of the TRIF-related adaptor molecule in association with TRIF by TLR4. The use of so many adaptors by TLR4 may explain the high versatility of this receptor in terms of cytokine induction.

The observation that TLR3 signaling depends primarily on a single adaptor, TRIF, may explain the fact that the array and amount of cytokines induced by poly(I:C) in DCs is somewhat reduced when compared with other TLR agonists such as LPS or peptidoglycan (46). Experimental evidence suggests that TLR2 and TLR5 cannot use the adaptors that belong to the MyD88-independent pathway (TRIF and TRIF-related adaptor molecule) and mostly rely on MyD88. Each TLR appears to possess a distinctive ability to activate DCs or PBMCs, suggesting that TLR-mediated responses cannot be simply catalogued as resembling either TLR2 (MyD88-dependent) or TLR3 (MyD88-independent) and that other signaling modalities may exist. These results also support the notion that various TLR agonists possess a different potential to affect T cell biology, either directly or through APC-derived cytokines.

As DCs express different subsets of TLR, fine-tuning of the inflammatory response to a pathogen at the intracellular signaling level appears to play an important role in the generation of pathogen-specific responses. Although the dissection of the signaling pathways activated by each TLR can only be achieved by studying each recognition event separately, cells that encounter complex pathogens such as bacteria are exposed to several microbial products simultaneously. Thus, the overall innate immune response will be a sum of each single stimulation.
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 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 maturation environment of DCs.

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