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The Toll-Like Receptor 5 Stimulus Bacterial Flagellin Induces Maturation and Chemokine Production in Human Dendritic Cells

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Toll-like receptors (TLRs) are pattern recognition receptors that serve an important function in detecting pathogens and initiating inflammatory responses. Upon encounter with foreign Ag, dendritic cells (DCs) go through a maturation process characterized by an increase in surface expression of MHC class II and costimulatory molecules, which leads to initiation of an effective immune response in naive T cells. The innate immune response to bacterial flagellin is mediated by TLR5, which is expressed on human DCs. Therefore, we sought to investigate whether flagellin could induce DC maturation. Immature DCs were cultured in the absence or presence of flagellin and monitored for expression of cell surface maturation markers. Stimulation with flagellin induced increased surface expression of CD83, CD80, CD86, MHC class II, and the lymph node-homing chemokine receptor CCR7. Flagellin stimulated the expression of chemokines active on neutrophils (IL-8/CXC chemokine ligand (CXCL)8, GRO-α/ CXCL1, GRO-β/CXCL2, GRO-γ/CXCL3), monocytes (monocyte chemoattractant protein-1/CC chemokine ligand (CCL)2), and immature DCs (macrophage-inflammatory protein-1α/CCL3, macrophage-inflammatory protein-1β/CCL4), but not chemokines active on effector T cells (IFN-inducible protein-10 kDa/CXCL10, monokine induced by IFN-γ/CXCL9, IFN-inducible T cell α chemoattractant/CXCL11). However, stimulating DCs with both flagellin and IFN-inducible protein-10 kDa, monokine induced by IFN-γ, and IFN-inducible T cell α chemoattractant expression, whereas stimulation with IFN-β or flagellin alone failed to induce these chemokines. In functional assays, flagellin-matured DCs displayed enhanced T cell stimulatory activity with a concomitant decrease in endocytic activity. Finally, DCs isolated from mouse spleens or bone marrows were shown to not express TLR5 and were not responsive to flagellin stimulation. These results demonstrate that flagellin can directly stimulate human but not murine DC maturation, providing an additional mechanism by which motile bacteria can initiate an acquired immune response. The Journal of Immunology, 2003, 170: 5165–5175.

Dendritic cells (DCs) represent a class of professional APCs that are critical in the initiation of a primary immune response. The proposed model for DC function is that the DCs take up foreign Ag in the periphery, process the Ag, and migrate to the T cell area of lymph nodes, where they present the Ag in the context of MHC along with costimulatory receptors, thereby activating naive T cells (1). Furthermore, innate immunity is thought to be triggered by pattern recognition receptors on APCs. Toll-like receptors (TLRs), which recognize conserved motifs on microorganisms, are thought to be largely responsible for this innate recognition. The TLR family consists of ten conserved germline-encoded type I transmembrane receptors that are present on APCs and function in response to triacylated lipopeptides (TLR1/2 heterodimers), diacylated lipopeptides (TLR2/6 heterodimers), dsRNA (TLR3), LPS (TLR4), flagellin (TLR5), imidazoquinolines (TLR7 and TLR8), and unmethylated CpG DNA (TLR9) (reviewed in Refs. 2–8).

In vitro studies of DC maturation have been conducted using cells derived from peripheral blood monocytes cultured in the presence of GM-CSF and IL-4. Such cells have an immature phenotype, having a high rate of endocytosis and expressing low levels of MHC class II, CD83, and the costimulatory molecules CD80 and CD86. Upon maturation, DCs down-regulate mechanisms of Ag capture, including endocytic activity and expression of Fc receptors, but they increase expression of costimulatory, adhesion, and MHC class II molecules (1, 9). Moreover, monocyte-derived DCs pulsed with tumor Ags ex vivo are a promising new tool in the treatment of malignant diseases (10, 11).

Recent work has shown DC maturation after exposure to microbial lipopeptides, LPS, viruses, and parasites (9, 12–20). Each of these microbes or their components has been found to activate cells through TLRs. This report demonstrates that human monocyte-derived DCs express TLR5, flagellin induces human DC maturation in vitro, and that this maturation is accompanied by chemokine and cytokine production.
Materials and Methods

**Reagents**

Human and murine IL-4 and GM-CSF were purchased from PeproTech (Rocky Hill, NJ). Human IFN-β was purchased from R&D Systems (Minneapolis, MN). LPS (Escherichia coli K12) was from Sigma-Aldrich (St. Louis, MO). LPS was purchased from contaminating lipopolysaccharides normally found in commercially available LPS preparations by double phenol extraction, exactly as described (21). Synthetic lipopolysaccharide/cell wall derivative (RS)-2,3-di[3-methylimidazol-1-yl]propyl]serine-lysine-OH is derived from an E. coli lipoprotein and was purchased from Bachem (Torrance, CA). Flagellin was purified from Salmo-

nella typhimurium (flex) as previously described (22). The flagellin preparation contained 20–25% of flagellin per microgram of protein as tested by Limulus amebocyte assay. This purified flagellin activates NF-κB in Chinese hamster ovary cells transfected with TLR5, but not with TLR2 or TLR4 (22). All other reagents were purchased from Sigma-Aldrich unless stated otherwise.

**Generation and maturation of DCs**

Buffalo coats were obtained from healthy volunteers and fractionated over Histopaque-1077. The PBMC layer was recovered and erythrocyte depletor was added to the RBC lysis buffer for 5 min at room temperature. The cell suspension was washed three times with 1× PBS (Invitrogen, Carlsbad, CA). The remaining adherent cells were then cultured in complete medium supplemented with GM-CSF and IL-4 (50 ng/ml each). On days 3 and 4, the DC cultures received an additional dose of GM-CSF and IL-4 (50 ng/ml each). On day 5, nonadherent DCs were harvested by gentle pipetting, counted, and plated in fresh medium containing GM-CSF and IL-4 (50 ng/ml each). On day 6, some DCs were matured by addition of 100 ng/ml LPS or 0.2–60 nM fliC for 1–48 h.

Primary human blood DCs were purified from human peripheral blood by immunomagnetic depletion of CD3-, CD11b-, and CD16-expressing cells followed by positive selection of CD4+ cells (Miltenyi Biotec, Auburn, CA).

Murine splenic DCs were isolated from spleens of C57BL/6 mice as previously described (23). Briefly, spleens were dissected into small pieces (1 mm3) and incubated at 37°C in complete RPMI 1640 supplemented with 2 mg/ml collagenase D for 45 min. Cell suspension was obtained by vigorous pipetting and passage through a 70-μm nylon mesh filter and was washed with complete medium. After lysis of RBCs, CD11c+ DCs were isolated using CD11c microbeads according to the manufacturer’s instructions (Miltenyi Biotec). The cells isolated were 90% CD11c+ as measured by FACS analysis.

Murine bone marrow-derived DCs were isolated from the marrow cavities of femurs and tibiae and were incubated in culture medium with 50 ng/ml each). On day 5, some DCs were matured by addition of 100 ng/ml LPS or 0.2–60 nM fliC for 1–48 h.

Primary human blood DCs were purified from human peripheral blood by immunomagnetic depletion of CD3-, CD11b-, and CD16-expressing cells followed by positive selection of CD4+ cells (Miltenyi Biotec, Auburn, CA).

**Flow cytometry**

Surface expression of various markers was assessed using CellQuest analysis software on a FACS Calibur (BD Biosciences, Mountain View, CA) flow cytometer. Surface expression was determined using the following FITC- and PE-conjugated Abs: CD86-FITC (Research Diagnostics, Flanders, NJ), CCR7-PE (R&D Systems), CD80-FITC, CD83-FITC, HLA-

DR-FITC, and CD11c-PE (BD PharMingen, San Diego, CA). The isotype control Abs were used accordingly in all experiments and were purchased from BD PharMingen. Human DCs were incubated in 1% human AB serum/PBS, and murine DCs were incubated with rat anti-CD16/CD32 (BD PharMingen) to block nonspecific binding. Murine CCR7 was detected using macrophage-inflammatory protein 3β (MIP-3β)/CC chemokine ligand (CCL) 19-Fc fusion protein provided by Jason Cyster (University of California, San Francisco, CA) (24). MIP-3β-Fc was detected by addition of biotin-conjugated goat anti-human Fc, followed by PE-conjugated streptavidin (Caltag Laboratories, Burlingame, CA).

**Quantitation of chemokines by ELISA**

Human IL-8/CXC chemokine ligand (CXCL) 8 (BioSource International, Camarillo, CA), IFN-γ-inducible protein 10 kDa (IP-10/CX chemokine ligand (CXCL) 10, IL-10, and IL-12 protein levels in the DC culture supernatants were measured by sandwich ELISA (R&D Systems).

**Results**

**Phenotype of iDCs**

It has been previously shown that LPS, lipopeptides, dsRNA, and unmethylated CpG DNA induce cellular activation via TLRs and that these pathogen-associated substances can induce DC maturation (9, 13, 15, 25–30). Flagellin, a conserved protein monomer that makes up the bacterial flagellar filament, is recognized through TLR5. Therefore, we decided to test whether flagellin could also induce maturation of DCs (22, 31–34). We used a common in vitro culture system for the production of human monocyte-derived DCs in which adherent PBMCs are cultured in IL-4 and GM-CSF for 5 days. As shown in Fig. 1A, day 0 adherent cells have surface expression of CD14, CD11c, and class II MHC, but not CD83, a surface phenotype consistent with these cells being monocytes (35). After culture of these cells in IL-4 and GM-CSF for 3 and 5 days, surface expression of CD14 decreases, whereas surface expression of CD11c increases, a change also reflected at the mRNA level.
Chemokine and cytokine expression in DC maturation

At the junction between innate and acquired immunity, DCs play an important role in initiating an immune response. The chemotaxis of effector cells to sites of infection and secondary lymphoid organs is an important aspect of immunity. To examine the potential role of DCs in trafficking of immune effector cells, we measured expression of chemokines (as well as cytokines) in iDC-DCs. We observed increased mRNA levels for a number of chemokines, notably growth-related oncogene-α (GRO-α)/CXCL1, GRO-β/CXCL2, GRO-γ/CXCL3, IL-8/CXCL8, monocyte chemoattractant protein-1 (MCP-1)/CCL2, MIP-1α/CCL3, and MIP-1β/CCL4, which are expressed quite early after stimulation (~1-3 h) at high levels. Interestingly, the T cell chemottractants IP-10/
CXCL10, monokine induced by IFN-γ (MIG)/CXCL9, and IFN-inducible T cell α chemoattractant (I-TAC)/CXCL11, which are expressed in LPS-DCs at later time points (~8 h), were not expressed in filC-DCs (Fig. 5, A and B).

As shown in Fig. 5C, filC stimulated the expression of the proinflammatory cytokines IL-1β and TNF, as well as the Th1 cytokines IFN-γ and IL-12 p40. In contrast with LPS-DCs, filC-DCs failed to induce expression of IFN-α and IFN-β. The inability of filC to induce IFN-α/β expression may explain why the IFN-inducible chemokines IP-10, MIG, and I-TAC were not expressed by filC stimulation. Recently, Vogel and coworkers (44) reported that TLR4, but not TLR2, agonists could induce IFN-β expression and subsequently STAT1α/β phosphorylation. In mice, LPS-induced IP-10 expression has been shown to be STAT1-dependent, as demonstrated by the failure of STAT1αβ−/− macrophages to express IP-10 mRNA in response to LPS (45). To determine whether other non-IFN-β-inducing stimuli also fail to induce IP-10, MIG, and I-TAC, we stimulated iDCs with synthetic lipopeptide (palmitoylcycteine[(R5)-2,3-di(palmitolxy)propyl]serine-lysine-OH, a TLR1/2 stimulus). Lipopeptide-stimulated DCs failed to express IFN-α/β as previously described and also failed to express IP-10, MIG, and I-TAC (data not shown). Thus, TLR5 (filC) and TLR1/2 (lipopeptide) agonists appear to similarly activate only a subset of TLR4 (LPS)-inducible proinflammatory genes.

To determine whether the increase in chemokine and cytokine mRNA expression is accompanied by protein secretion, ELISA was performed on the supernatants of filC-DCs. We observed increased IL-8 in the supernatant of filC-DCs compared with that of iDCs, whereas IP-10 protein production was not detected in filC-DCs (Fig. 5, E and F). This pattern of chemokine secretion induced by flagellin is similar to what has been previously described for macrophages stimulated with lipopeptide (44).
Upon encounter with microbial pathogens, DCs produce immunostimulatory cytokines, such as IFN-γ and IL-12, which are important for selective activation of T cells into the Th1 phenotype. Interestingly, LPS-stimulated DCs, but not flagellin-stimulated DCs, produced bioactive IL-12 p70 (Fig. 5G). Meanwhile, neither LPS nor flagellin induced IL-10 protein production (Fig. 5H). These data fit with previously published data by Thoma-Uszynski et al. (12), which demonstrate that LPS and lipopeptide induced IL-12, but not IL-10, in monocyte-derived DCs (10). These data suggest that flt-DCs may not be as potent at generating a Th1 cytokine response as LPS-DCs or lipopeptide DCs.

Exogenous human IFN-β restores TLR5-mediated gene expression

LPS, but not flagellin, induced the expression of IP-10, MIG, and I-TAC in a time-dependent manner (Fig. 5, A and B). Treatment of DCs with flagellin (20 nM) in combination with rIFN-β (50, 25, and 10 U/ml) induces expression of IP-10, MIG, and I-TAC mRNA, whereas stimulation with IFN-β or flagellin alone failed to induce these chemokines (Fig. 6, A–C). This suggests that IFN-β production after LPS stimulation provides an additional signal required for IP-10, MIG, and I-TAC expression. Meanwhile, addition of IFN-β had no effect on flagellin-induced IL-8 mRNA expression (Fig. 6D). To determine whether the increase in IP-10 message level was accompanied by protein secretion, ELISA was performed on the supernatants of flagellin and IFN-β-stimulated DCs. As shown in Fig. 6E, treatment of DCs with flagellin in combination with rIFN-β induced IP-10 protein production.

Bacterial flagellin fails to mature murine DCs

To demonstrate TLR5 function in murine DCs, we isolated murine splenic DCs and stimulated them with fltC. Surprisingly, purified bacterial flagellin was unable to mature murine splenic DCs in vitro as determined by cell surface and mRNA expression of CD80, CD86, and CCR7 (Fig. 6, A and B). As shown in Fig. 6C, QPCR analysis of murine DCs revealed no expression of TLR5, consistent with their inability to respond to flagellin. Interestingly, murine bone marrow-derived DCs, peritoneal macrophages, and the murine macrophage cell line RAW 264.7 fail to express TLR5 mRNA (Fig. 6D). We were only able to demonstrate positive TLR5 expression in murine skin epithelia (Fig. 6D). These results are in agreement with a recent report by Applequist et al. (46), which also used mouse skin cDNA as a positive control and demonstrated little to no expression of TLR5 in several murine monocyte, macrophage, and DC cell lines. Also, we detected little or no TLR5 mRNA expression in resident and thioglycollate-elicited...
peritoneal macrophages from C57BL and BALB/c mice. Furthermore, these macrophages, as well as purified splenic DCs, failed to produce TNF or IL-8 protein production in response to flagellin stimulation (data not shown).

Interestingly, human DCs express TLR5, but not TLR9 (Fig. 1C), whereas mouse DCs express TLR9, but not TLR5 (Fig. 6C). The biological consequence of this species-specific difference remains unclear and will be discussed below.

FIGURE 5. Chemokine and cytokine expression during flagellin-induced DC maturation. Total RNA was isolated from day 5 iDCs (1 x 10^6 cells) stimulated with 20 nM flIC or 100 ng/ml LPS for 1, 3, 8, 24, and 48 h. Expression of chemokines (A and B) and cytokines (C and D) was quantified by QPCR. The number of transcripts is normalized to the number of copies of GAPDH. Data shown are representative of three experiments. E–H, The concentrations of the chemokines and cytokines IL-8, IP-10, IL-12 p70, and IL-10 in the above cultured supernatants were measured by ELISA. The results shown are from a single experiment with a single donor and are representative of three similar experiments.
Discussion
In this report, we demonstrate maturation of human monocyte-derived DCs to the TLR5 stimulus bacterial flagellin. This observation helps explain previous reports regarding the high antigenicity and adjuvant activity of bacterial flagellin (47). Our results demonstrate that TLR5 is expressed on human iDCs and that these cells are sensitive to flagellin stimulation, consistent with other reports demonstrating TLR5 expression in human monocytes and iDCs by Northern blot and RT-PCR analysis (37, 38, 48).

We demonstrated a dose-dependent induction of DC maturation using 0.2–60 nM flC. In subsequent experiments we chose to present data using 20 nM = 1 μg/ml flC, because it was the lowest concentration that consistently gave a robust response and was equivalent to the potent maturation inducer LPS. Flagellin (20 nM) and LPS (100 ng/ml) consistently induced DC activation as measured by T cell stimulatory activity, induction of maturation and costimulatory molecules, reduction in endocytic activity, and equivalent expression of TNF and IL-1β (Fig. 5B). However, we have consistently found that LPS induces higher levels of CD86 on LPS-DCs, which may explain the slightly higher T cell stimulatory activity of LPS-DCs compared with flC-DCs (Figs. 2B and 4).

Interestingly, our observations suggest that there are differences between DCs matured with LPS and DCs matured with flagellin. flC-DCs do not express the T cell chemokines MIG, IP-10, and I-TAC or IFN-β, which are all expressed by LPS-DCs (Fig. 7). This suggests that there are qualitative differences between the signals generated by TLR5 compared with that provided by TLR4, a notion consistent with recent reports demonstrating the role of IFN regulatory factor 3 (IRF3) in mediating TLR3- and TLR4-specific transcriptional responses, but not TLR2 and TLR9 responses (49, 50). The simplest explanation for the chemokine disparity between flC-DCs and LPS-DCs is that LPS stimulation results in TLR4-mediated early IFN-β secretion through an IRF3-mediated signaling pathway, leading to the expression of MIG, IP-10, and I-TAC. TLR5-mediated signals do not result in this early IFN-β production and consequently do not mediate the expression of MIG, IP-10, and I-TAC (Figs. 5, A and C, and 8). To
test this hypothesis, we stimulated DCs with flagellin in combination with exogenous rIFN-β. Interestingly, stimulation with both flagellin and IFN-β resulted in IP-10, MIG, I-TAC expression, whereas flagellin or IFN-β treatment alone failed to induce these chemokines (Fig. 6). These data suggest that IFN-β production after LPS stimulation provides an additional signal required for IP-10, MIG, and I-TAC expression. The biological consequence of this disparity between the LPS and flagellin responses is not clear; however, in an actual infection, a number of different TLRs are likely to be engaged simultaneously, perhaps making the specific signaling pathways used by any one TLR biologically less relevant (2, 4).

Flagellin and LPS induced the expression of a number of other chemokines. The neutrophil-active chemokines GRO-α, GRO-β, GRO-γ, and IL-8 were expressed in both LPS- and flagellin-treated DCs, as were the monocyte/macrophage/NK-active chemokines MCP-1, MIP-1α, MIP-1β, and RANTES. These findings suggest that DCs not only play a role in the initiation of an acquired immune response, but that they may also play a role in amplifying the early immune response by participating in the trafficking of innate immune cells to sites of infection. The early expression of these chemokines (1–3 h) is consistent with the notion that iDCs, upon encounter with pathogens, express these chemokines before the expression of the lymph node homing chemokine receptor CCR7, which is maximally expressed at 24 h after stimulation (Fig. 2B).

Another intriguing finding was that murine splenic DCs do not mature to purified bacterial flagellin. Besides providing a control for the purity of our flagellin preparation, this seems at odds with the recent findings by McSorley et al. (47) demonstrating the ability of bacterial flagellin to effectively elicit a robust CD4 T cell response in vivo. Though our in vitro findings predict flagellin will not directly stimulate DC maturation in vivo, it is still possible that other cells, such as skin epithelia and gut epithelia, which express TLR5 (Fig. 7D), are able to produce cytokines (such as TNF and IFNs), which may then trigger DC maturation (34, 51). This is consistent with our previous data demonstrating IL-6 production in the serum of flagellin-injected wild-type mice, but not of MyD88 knockout mice (20).

A recent report by Renshaw et al. (52) demonstrated murine TLR5 expression in thioglycollate-elicited peritoneal macrophages from 2- to 3-mo-old C57BL/6 mice. Moreover, this group found...
FIGURE 8. Model of TLR5- vs TLR4-induced chemokine production in human DCs. Activation of TLR5 and TLR4 by flagellin and LPS, respectively, induces a differential set of chemokines. Both TLR5 and TLR4 agonists induced the expression of chemokines active on neutrophils (IL-8/CXCL8, GRO-α,β,γ/CXCL1–3), monocytes (MCP-1/CCL2), NK cells (RANTES/CCL5), and iDCs (MIP-1α/CCL3, MIP-1β/CCL4), but only the TLR4 agonist LPS induced chemokines active on effector T cells (IP-10/CXCL10, MIG/CXCL9, I-TAC/CXCL11). TLR4 and TLR3 agonists have been shown to induce nuclear translocation of IRF3, which leads to the expression of IFN-β. IFN-β stimulates the IFN-α/β receptor through an autocrine loop on DCs, which leads to the phosphorylation of STAT1α/β. STAT1α/β then induces expression of the T cell attracting chemokines IP-10, MIG, and I-TAC. Stimulation with flagellin in combination with exogenous rIFN-β induces IP-10, MIG, and I-TAC, whereas stimulation with IFN-β or flagellin alone fails to induce these chemokines. This suggests that IFN-β production after LPS stimulation provides an additional signal required for IP-10, MIG, and I-TAC expression.

that flagellin treatment could induce IL-6 and TNF production from thioglycollate-elicited peritoneal macrophages isolated from young mice, but not old mice. In addition, splenic macrophages were found to express lower levels of TLR5 and were nonresponsive to flagellin treatment. In Fig. 7, we demonstrated that resident peritoneal macrophages did not express TLR5 mRNA. We also assessed TLR5 mRNA expression in resident and thioglycollate-elicited peritoneal macrophages from C57BL/6 and BALB/c mice. Unlike Renshaw et al. (52), we were only able to detect low levels of TLR5 mRNA expression in these peritoneal macrophages (Fig. 7; data not shown). Furthermore, these cells did not produce TNF or IL-8 in response to flagellin stimulation (data not shown). These differences may be due to the way the cells were handled or cultured. Moreover, Renshaw et al. (52) did not provide any information about the purity of their flagellin preparations. We have found that it is very difficult to eliminate TLR2/TLR4 agonists from flagellin preparations. Our flagellin preparations are purified using extensive dialysis and size fractionation and are rigorously tested on Chinese hamster ovary cells stably transfected with TLR2, TLR4, or TLR5. Only flagellin preparations/fractions that solely activated TLR5-expressing cells were used in our studies. Alternatively, Renshaw et al.’s flagellin preparation (52) may be modified, enabling it to stimulate through murine TLR5 or in combination with another unidentified receptor. Indeed, we have found that monomeric flagellin (induced by sonicating) stimulates DCs more potently than does aggregated flagellin. However, both of these forms failed to activate murine macrophages or murine DCs in our experiments. Interestingly, Renshaw et al. (52) reported that splenic macrophages expressed only low levels of TLR5 and did not respond to flagellin, whereas thioglycollate-elicited peritoneal macrophages expressed higher levels of TLR5 and responded to flagellin. This might indicate that TLR5 varies based on the activation state (thioglycollate elicited) and anatomical location (spleen vs peritoneum). However, we have not been able to reproduce these findings.

The biological significance of murine DC unresponsiveness to flagellin is unclear. Again, a number of different TLRs are likely stimulated during exposure to microbes and the stimulation of TLR5 by flagellin is just one of these possible interactions. Therefore, it is unlikely that the lack of recognition of bacterial flagellin by murine DCs will result in mice displaying enhanced susceptibility to flagellated bacteria compared with humans; however, it may lead to qualitative differences in the immune response to flagellated bacteria between mice and humans (53, 54).

Recently, Rehli (reviewed in Ref. 54) clarified the species-specific variations of TLR expression in mice and humans. Recent data from several groups suggest that constitutive and inducible TLR expression in different cell types and in different species are controlled by transcriptional regulation (55–57). Moreover, gene regulatory elements found in the proximal promoters of TLR genes control cell-type specificity and inducible TLR expression in mice and humans. For example, the human TLR2 gene is constitutively expressed in monocytes and is not inducible by microbial pathogens. In contrast, the mouse TLR2 gene, which has NF-κB and
STAT5 transcription factor binding sites in its proximal promoter, is rapidly induced by microbial stimuli (57). The human TLR3 gene is constitutively and selectively expressed in myeloid DCs, whereas the mouse TLR3 gene is inducible and expressed in macrophages. Likewise, TLR9 and TLR5 appear to be regulated in a cell type- and species-specific manner. Human DCs express TLR5, but not TLR9 (Fig. 1C), whereas mouse DCs express TLR9, but not TLR5 (Fig. 7C). The biological consequence of this species-specific difference remains unclear.

Our findings and other studies suggest that the stimulation of any TLR expressed on DCs is sufficient for maturation, as measured by surface expression of coagonitors, T cell stimulatory activity, and endocytic activity (9, 38). Finally, we demonstrate a qualitative difference in gene expression of IFN-α/β and the T cell chemokines IP-10, I-TAC, and MIG between iC-DCs and LPS-DCs.

References


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