Toll-like Receptor 2 (TLR2) and TLR4 Differentially Activate Human Dendritic Cells*

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Toll-like receptors (TLRs) mediate cell activation by various microbial products. Here, we demonstrate that activation of dendritic cells by TLR2 or TLR4 agonists, although it led to comparable activation of NF-κB and mitogen-activated protein kinase (MAPK) family members, resulted in striking differences in cytokine and chemokine gene transcription, suggesting that TLR2 and TLR4 signaling is not equivalent. A TLR4 agonist specifically promoted the production of the Th1-inducing cytokine interleukin-12 (IL-12) p70 and the chemokine interferon-γ inducible protein (IFN-γ)-10, which is also associated to Th1 responses. In contrast, TLR2 stimulation failed to induce IL-12 p70 and interferon-γ inducible protein (IFN-γ)-10 but resulted in the release of the IL-12 inhibitory p40 homodimer, producing conditions that are predicted to favor Th2 development. TLR2 stimulation also resulted in preferential induction of IL-8 and p19/IL-23. Involvement of phosphatidylinositol-3-kinase and p38 MAPK in the TLR-mediated induction of several cytokine and chemokine messages was demonstrated using specific inhibitors. Thus, TLRs can translate the information regarding the nature of pathogens into differences in the cytokines and chemokines produced by dendritic cells and therefore may contribute to the polarization of the acquired immune response.

Cells of the innate immune system possess germ line-encoded receptor molecules that enable them to recognize structural components conserved among classes of microorganisms (1, 2). This recognition event serves two purposes. First, it alerts the immune system to the presence of pathogens so that an immediate response can be mounted to contain the infection. Later, during the establishment of acquired immunity, this recognition event is thought to provide information regarding the nature of the invading microorganism that will contribute to the determination of Th helper (Th)1 cells into either Th1 cells, which promote cell-mediated immunity, or Th2 cells, which encourage humoral responses. Cytokines play a pivotal role in this process, with IL-12 committing cells to Th1 lineage differentiation and IL-4, in the absence of IL-12 and IFN-γ, promoting Th2 development (3). However, the mechanism by which the nature of the pathogens is translated into differences in the cytokines produced remains poorly understood.

Recognition of several microbial products is mediated by members of the Toll-like receptor (TLR) family (4, 5). These receptors are characterized by an extracellular leucine-rich domain that shows considerable divergence and is probably involved in recognition of different agonists, and a highly conserved cytoplasmic Toll-IL-1 receptor domain that connects the receptor to the signaling machinery shared by IL-1 and IL-18 (6, 7). Although all TLRs for which an agonist has been identified appear to activate similar signaling pathways, including NF-κB, p38 MAPK, stress-activated protein/JNK, and MAPK (Erk1/2) kinases (8–12), it is not clear whether different TLRs differ in their ultimate function, the activation of innate immune responses. Evidence that TLRs are not functionally equivalent is accumulating. Homodimerization of the cytoplasmic domain of TLR4 is sufficient to initiate signaling that leads to TNF-α production by a macrophage cell line. In contrast, heterodimerization of TLR2 cytoplasmic domain with either TLR1 or TLR6 cytoplasmic tails is required for induction of this cytokine (13). These findings indicate that despite their high sequence homology, the cytoplasmic tails of different TLRs are not functionally equivalent and suggest that different signals may emanate from distinct receptors. The concept that distinct TLR may differ in their signaling ability is also supported by studies of Drosophila TLRs. Despite sharing homologous cytoplasmic domains, toll and 18 wheeler, which mediate the immunity of the fly to fungi and bacteria, respectively, are known to activate very different and nonoverlapping gene expression (14, 15). In this study, we explored the possibility that TLR2 and TLR4 may differentially activate human DCs.

EXPERIMENTAL PROCEDURES

Reagents—LPS (Escherichia coli K12 L2525) was from List Biological Laboratories (Campbell, CA). It was purified from contaminant lipopolysaccharide and was purchased from Bachem (Torrance, CA). OspB-L is a synthetic lipopeptide (Cys-Ala-Gln-Lys-Gly-Ala) derived from Fluka (Milwaukee, WI). Zymosan was from Sigma. Synthetic lipopeptide Pam3-Cys-Ala-Gly is derived from an E. coli lipoprotein and was purchased from Bachem (Torrance, CA). OspB-L is a synthetic lipopeptide (Cys-Ala-Gln-Lys-Gly-Ala) derived from Borrelia burgdorferi, and OspB is the non-acylated hexapeptide that served as a control as it possesses no biological activity. They were both kindly provided by Dr. Juan C. Salazar, University of Connecticut Medical School. LY-294,002 and SB203580 were purchased from Alexis Biochemicals (San Diego, CA). U0126 was purchased from Cell Signaling Technology (Beverly, MA). Plasmids and Cell Lines—TLR2 and TLR4 sequences were gener-
ated by reverse transcription-polymerase chain reaction from human monocyte total RNA using Superscript II reverse transcriptase (Life Technologies, Inc.) and PfuTurbo DNA polymerase (Stratagene, La Jolla, CA) using the following primers: TLR4–5’/H11032, GCGCGGCGGCCGCGGAAAGCTGGGAGCCCTGCGTGGAGG; TLR4–3’/H11032, GCGCGGATCCTCAGATAGATGTTGCTTCCTGCC; TLR2–5’/H11032, GCGCGGCGGCCGCGGAAGAATCCTCCAATCAGGC; and TLR2–3’/H11032, GCGCGGATCCCTAGGACTTTATCGCAGCTCTCAG. The TLR2 sequence was cloned in the NotI-BamHI sites of pFLAG-CMV-1 (Sigma). TLR4 sequence was cloned as a NotI-BamHI fragment into a modified pFLAG-CMV-1 that contained the c-Myc epitope tag in place of the FLAG tag. C-terminal FLAG-tagged MD-2-containing vector was kindly provided by Dr. Miyake (Saga Medical School). The MD-2 sequence was excised as a XhoI-NotI fragment and inserted in the SalI-NotI sites of vector pBOS-H2Bgfp, which also contains the blastidicin resistance gene. A subclone of the HeLa cell line was transcribed using Superscript II reverse transcriptase (Life Technologies, Inc.) with pFLAG-CMV-TLR2 and pPuro or with pMyc-CMV-TLR4 and pBOS-MD-2, and stable transformants were selected in puromycin or blastidicin containing medium, respectively. Several colonies were isolated, and TLR2 or TLR4 expression was tested by Western blot and FACScan analysis using M2-FLAG or Myc monoclonal antibodies. Two clones with comparable TLR2 and TLR4 expression were expanded and further characterized.

**Dendritic Cells**—Human peripheral blood mononuclear cells were isolated from Leukopacks (courtesy of the Kraft Blood Donors Center) by routine Ficoll-Hystopaque density gradient centrifugation. Monocytes were purified from human peripheral blood mononuclear cells using MACS CD14 microbeads (Miltenyi Biotech, Auburn, CA) according to the manufacturer’s recommendation. Purity was checked by staining with a FITC-conjugated anti-CD14 antibody (Sigma) and FACScan analysis and was routinely found to be greater than 94%. Immature DCs were obtained by incubating monocytes at 1/10^6/ml in RPMI 1640 medium supplemented with 10% fetal calf serum and recombinant human (rh) granulocyte macrophage colony-stimulating factor (10 ng/ml) and rhIL-4 (10 ng/ml) (both from R&D Systems, Minneapolis, MN) for 8 days. Fresh complete medium was replaced every 4 days. rhIFN-γ was from R&D Systems. Recombinant murine TNF-α was from Roche Molecular Biochemicals. rhIL-1β was kindly provided by Marta Muzio (Mario Negri Institute, Milan, Italy).

**Cells Staining**—DC surface markers were stained using the following PE- or FITC-conjugated mAbs from Pharmingen (San Diego, CA): phycoerythrin-CD1a (clone HI149), phycoerythrin-CD4 (clone 5C3), phycoerythrin-CD83 (clone HB15e), FITC-CD86 (clone 2331), and FITC-human leucocyte antigen-DR (clone G46–6). FITC-CD14 (clone UCHM1) was from Sigma.

**Luciferase Assay**—HeLa-TLR2 or HeLa-TLR4/MD2 cells were transiently transfected in six-well plates using Superfect reagent (Life Technologies, Inc.) with 0.5 μg of endothelial leucocyte adhesion molecule-
FIG. 2. TLR2 and TLR4 agonists induce similar phenotypic maturation of human DCs. DCs were stimulated with LPS (10 ng/ml) or PGN (10 μg/ml) for 36 h. Staining of surface markers was analyzed by FACScan flow cytometry. Dashed line and thick line represent LPS and PGN treatment, respectively. Shaded areas represent staining of the untreated cells, and outlined areas represent isotype control Abs.

FIG. 3. TLR4 but not TLR2 agonists induce IP-10 production in human immature DCs. A, RNase protection assay analysis of transcripts for chemokines. DCs were stimulated with TLR2 or TLR4 agonists for 4 h. Two representative experiments are shown. Lines connect the unprotected probe fragments to the corresponding protected mRNA species. OspB is the non-acylated hexapeptide that serves as a control as it possesses no biological activity. The smeared bands in this sample lane are due to RNA degradation. B, DCs were stimulated with LPS (10 ng/ml), PGN (10 μg/ml), or Pam-Cys (10 μg/ml) for 36 h. IP-10 was measured in supernatant by ELISA. Measurements were done in duplicate. Data (mean ± S.D.) are from three donors. The detection limit of the assay was 31 pg/ml. C, RNase protection assay analysis of transcripts for chemokines in HeLa. HeLa-TLR2 and HeLa-TLR4/MD-2 were stimulated with LPS (10 ng/ml), PGN (10 μg/ml), or zymosan (50 mg/ml) for 4 h.
luciferase and 1 μg of pcDNA-CD14 (kindly provided by Dr. Golenbock, Boston University) and 0.1 μg of CMV-β-galactosidase. Luciferase assay was performed using Promega (Madison, WI) reagents according to the manufacturer's recommendations. Efficiency of transfection was normalized by measuring β-galactosidase in cell lysates.

**EMSA**—10^6 DCs were lysed in hypotonic buffer (10 mM Hepes, pH 7.5, 10 mM KCl, 0.5% Nonidet P-40, 0.1 mM EDTA, 1 mM EGTA, 1 mM DTT, 1 mM phenylmethylsulfonyl fluoride) by vigorous vortexing. Lysates were spun at 8000 rpm for 5 min, and the nuclear pellets were resuspended in 20 mM Hepes, pH 7.5, 400 mM NaCl, 1 mM EDTA, 1 mM EGTA, 1 mM DTT, 1 mM phenylmethylsulfonyl fluoride. 6 μg of nuclear protein extract were incubated for 30 min at room temperature with 32P-labeled probe (CAGAGGGGACTTTCCGAG) and 2.5 μg of poly(dI-dC) in 10 mM Hepes, pH 7.5, 0.5% Triton, 100 mM NaCl, 2.5% glycerol, 2 mM DTT, 1 mM bovine serum albumin. The DNA-protein complex was resolved by PAGE, and the dried gel was exposed for autoradiography.

**Jun Kinase Assay**—Cells were lysed in 25 mM Hepes, pH 7.5, 300 mM NaCl, 0.1% Triton, 0.2 mM EDTA, 1.5 mM MgCl₂, 20 mM β-glycerophosphate, 1 mM Na3VO4, 0.5 mM DTT, and 1 μM phenylmethylsulfonyl fluoride. Cell lysates containing 100 μg of proteins were incubated for 3 h at 4°C with glutathione-agarose beads coated with glutathione S-transferase-c-Jun recombinant protein. Beads were then washed three times with lysis buffer and twice with kinase buffer (20 mM Hepes, pH 7.5, 20 mM β-glycerophosphate, 10 mM FNP, 10 mM MgCl₂, and 10 mM DTT). The kinase reaction was performed at 37°C for 30 min in kinase buffer supplemented with 10 μM ATP and 5 μCi of [γ-32P]ATP per sample. Proteins were resolved by PAGE, and the dried gel was exposed for autoradiography. Phospho-MAPK antibodies were from Cell Signaling (Beverly, MA).

**RNase Protection Assay**—Total RNA was isolated using Trizol reagent (Life Technologies, Inc.). RNase protection assay was performed using 4–6 μg of total RNA using the BD Biosciences-PharMingen Ribonuclease kit according to the manufacturer's recommendations. The hCK-2b, hCK-5, and hCK3 multiprobe template sets were used. The templates for IL-23 p19 and for GAPDH were generated by reverse transcription-polymerase chain reaction using the following primers: IL-23a, GCAGAGCTGTAATGCTGCTG; IL-23b, CCGATCCTAGCAGCTTCTC. The polymerase chain reaction product was cloned into pCR2.1-TOPO, and the p19 sequence was excised by EcoRI-BamHI restriction and cloned into the EcoRI-BamHI sites of pcDNA3 (Invitrogen, Carlsbad, CA). For GAPDH, the following primers were used: GAPDHa, CGCGCTCGAGCCCAGAAGACTGTGGATGG; GAPDHb, CGCGGAATTCGGCAGGTTTTTCTAGACGG. The polymerase chain reaction product was digested with XhoI-EcoRI and cloned into the EcoRI-XhoI sites of pcDNA3. The templates were linearized by EcoRI or XhoI restriction, phenol-extracted, and precipitated, and 100 ng were used in a standard RNase protection assay.

**ELISA**—DCs were plated in RPMI 1640 medium/10% fetal calf serum at a concentration of 2 × 10^6 cells/ml in 48-well plates and stimulated as described in the figure legends. IP-10, IL-8, IL-12 p70, and IL-12 p40 were measured by ELISA using matched pairs of antibodies from R&D Systems according to the manufacturer's recommendations. The IL-12 p40 ELISA is specific for the p40 subunit with less than 5% cross-reactivity with the IL-12 p70.

**RESULTS**

To determine whether TLR2 and TLR4 differ in their ability to activate DCs, several microbial products that have been previously demonstrated to activate different TLRs were tested. Highly purified E. coli LPS was used as TLR4 agonist.
S. aureus PGN (19, 20) and yeast zymosan (21) were used as agonists of the TLR2/TLR6 heterodimer (13). In addition, two synthetic lipopeptides derived from bacterial lipoproteins, OspB-L and Pam-Cys, were used. Similar peptides have been shown to activate cells through TLR2 paired to a yet unidentified TLR (22, 23). Preliminary experiments were conducted in order to confirm specificities and characterize dose responses using HeLa clones stably transfected with TLR2 or TLR4/MD-2. Increasing concentrations of zymosan, PGN, and OspB-L and Pam-Cys lipopeptides were able to activate NF-κB-dependent luciferase production in HeLa-TLR2 but not in HeLa-TLR4/MD-2 (Fig. 1A). As expected, the activation of NF-κB by LPS was detected only in HeLa-TLR4/MD-2. This response was MD-2-dependent (data not shown) and was not detected in HeLa-TLR2 even at concentrations several logs higher than those that elicited a maximal response in HeLa-TLR4/MD-2. Similar doses of LPS or PGN were able to activate NF-κB to the same degree in human immature DCs, as assessed by EMSA (Fig. 1B). Supershift experiments demonstrated that for both agonists, the main NF-κB species induced was a p65/p50 dimer (data not shown). In addition to NF-κB activation, LPS and PGN are known to activate p38 MAPK, stress-activated protein/JNK, and MAPK (Erk1/2) kinases in a TLR-dependent fashion. The activation state of these kinases was tested in DCs treated with LPS and PGN. In these cells, both LPS and PGN activated stress-activated protein/JNK (Fig. 1C), MAPK (Erk1/2) (Fig. 1D), and p38 MAPK (Fig. 1E) to the same extent. MAPK (Erk1/2) activation was also similarly affected by both agonists in HeLa-TLR2 and HeLa-TLR4/MD-2 (data not shown). Finally, we compared the effects of these microbial products on the induction of maturation of DCs (Fig. 2). Treatment with either LPS or PGN markedly induced equivalent expression of mature DC surface markers CD40, CD83, CD86, and human leukocyte antigen-DR. Thus, optimal and comparable cell activation in HeLa and DCs occurred at doses of 1–10 ng/ml LPS, 10 μg/ml PGN, 50 μg/ml zymosan, 2 μg/ml lipopeptide Pam-Cys, and 10 μg/ml lipopeptide OspB-L.

Activation of cells by microbial products through TLR2 and
TLR4 is known to induce the secretion of various cytokines and chemokines. Therefore, we investigated whether TLR2 or TLR4 agonists differentially regulate the expression of these factors in human immature DCs. The pattern of expression of several chemokines, including Ltn, RANTES, IP-10, MIP1α, MIP1β, MCP-1, IL-8, and I-309, was analyzed using RNase protection assays (Fig. 3A). Housekeeping genes L32 and GAPDH provided internal controls. Whereas the majority of the chemokines were induced to the same extent by both TLR2 and TLR4 agonists after 4 h of stimulation, one transcript, the message for IP-10, was specifically induced by LPS but not by the TLR2 agonists PGN, zymosan, or lipopeptides. This effect was consistently observed using DCs derived from several donors and was seen at different time points and for all the effective doses of agonists tested. The lowest effective dose of LPS (0.1 ng/ml) was still able to induce IP-10 transcript, whereas maximally potent doses of PGN and zymosan never induced IP-10. The IP-10 message was not observed even after 18 h of stimulation with PGN (see Fig. 4) or zymosan and with prolonged overexposure of the autoradiograph. Lipopolysaccharide-stimulated DCs secreted large quantities of IP-10 protein in the media (Fig. 3B). This chemokine was completely absent in PGN-stimulated, DC-conditioned media. IP-10 message induction by TLR4 agonist was confirmed in HeLa TLR4/MD-2 (Fig. 3C), thus ruling out the possibility that the induction of this transcript, rather than being TLR4-mediated, was indirectly stimulated by IFN-γ released by few contaminating cells.

In some experiments, TLR2 stimulation appeared to preferentially induce the transcripts of specific chemokines, such as IL-8 and MIP-1α. Initially, we interpreted this effect as a result of the supramaximal concentration of TLR2 agonist used to rule out the possibility that insufficient cell stimulation was the cause of the lack of IP-10 transcript. Surprisingly, when RNAs of cells stimulated for 14 h (instead of 4 h, as in previous experiments) with optimal agonist concentrations were analyzed, the IL-8 transcript was detected exclusively in cells stimulated with TLR2 agonist (Fig. 4A). This 4-h stimulation resulted in comparable IL-8 message induction, whereas during prolonged stimulation, IL-8 message was detected exclusively in cells stimulated with TLR2 agonists. Note that in the HeLa cell lines, 4-h stimulation also resulted in comparable level of IL-8 transcript (Fig. 3C). Measurement of IL-8 in DC culture media revealed that cells stimulated for 24 h with TLR2 agonist released 100 times more IL-8 than cells stimulated with TLR4 agonist (400 ± 32 ng/ml versus 4.4 ± 0.26 ng/ml) regardless of the concentration of agonist used (Fig. 4B).

To test whether stimulation through TLR2 or TLR4 also resulted in differences in the expression pattern of proinflammatory cytokines, transcript levels for IL-12 p35 and p40 subunits, IL-10, IL-1α and IL-1β, IL-1ra, IL-6, IL-18, and IFN-γ were analyzed (Fig. 5A). As was observed for chemokine expression, most cytokine transcripts were induced to the same extent by both TLR2 and TLR4 agonists, but one transcript, IL-12 p35, was specifically induced only by the TLR4 agonist LPS. Similarly to IP-10, IL-12 p35 message was not observed even at supramaximal doses of PGN or zymosan or for prolonged incubation times (18 h). Interestingly, the transcript of IL-12 p40 subunit was equally induced by both TLR agonists. This protein can form homodimers that have been shown to act as an IL-12 receptor antagonist (24). Measurements of IL-12 p70 and IL-12 p40 protein in the cell culture supernatants of stimulated DCs confirmed the RNase protection data (Fig. 5B). Interleukin-12 p70 was produced at low but detectable levels only in the media from cells stimulated with LPS. In contrast, IL-12 p40 was produced at comparable levels in the media of cells stimulated with either LPS or PGN. Note that in these experiments, IL-12 p40 was not produced in such excess of IL-12 p70 to be able to block its activities. In fact, for this to happen, a 500–1000-fold excess of p40 should be produced (24). Optimal IL-12 p70 production is known to require IFN-γ priming. Cotreatment of DCs with IFN-γ restored the ability of PGN and lipopeptides to induce IL-12 p70, as previously reported by others (29). However, even under these extreme conditions, LPS appeared to be a more potent stimulus for IL-12 p70 secretion. It should be noted that the primary encounter between a DC and a microbe is likely to occur in an IFN-γ-poor environment. Under these conditions, the low amounts of IL-12 p70 induced by TLR4 agonist might be enough to initiate the positive feedback loop between IL-12 and IFN-γ, thus producing conditions that would favor Th1 lineage commitment.

It has recently been shown that IL-12 p40 can pair to a protein called p19 to form IL-23, a novel cytokine with biolog-

**Fig. 6.** TLR2 agonist is a more powerful IL-23 p19 inducer than TLR4 agonist. RNase protection assay analysis of transcripts for IL-23 p19. DCs were stimulated with LPS (10 ng/ml) or PGN (10 μg/ml) for 4 h.

**Fig. 7.** TLR4 agonist is a more powerful IFN-β inducer than TLR2 agonist. RNase protection assay analysis of transcripts for proinflammatory cytokines. DCs were stimulated with TLR2 or TLR4 agonists for 4 h.
ical activities similar but not identical to those of IL-12 (25). We therefore analyzed the expression of p19 using RNase protection assay (Fig. 6). Surprisingly, we found that the message for p19 was preferentially induced by TLR2 agonist. The reagents to test whether the p19 message is translated into protein and whether bioactive IL-23 is formed are at present not available.

Using the same experimental approach, we next analyzed the pattern of expression of more proinflammatory cytokines, including TNF-$\alpha$, IFN-$\gamma$, transforming growth factor-$\beta_1$, transforming growth factor-$\beta_2$, and transforming growth factor-$\beta_3$. Again, we found that the message of one of these cytokines, IFN-$\gamma$, was preferentially induced by TLR4 agonist (Fig. 7).

In order to investigate the molecular mechanism responsible for the differences in cytokines and chemokines gene induction observed we used pharmacological inhibitors of MAPK/Erk1/2, of p38MAPK, and of PI3K. As shown in Fig. 8 induction of chemokine and cytokine transcripts were most potently blocked by LY-294002, an inhibitor of PI3K, and by SB203580, an inhibitor of p38MAPK, whereas MAPK(Erk1/2) pathway inhibitor U0126 (or PD98059, data not shown) was less effective.

**DISCUSSION**

Once activated by microbial products, DCs start producing a variety of soluble factors that direct T helper cell differentiation toward the type of immune response, humoral versus cellular-mediated, that is better suited to fight the invading pathogen. The mechanism by which the nature of the pathogen determines the panoply of factors produced by DCs, and therefore the type of adaptive immune response, is poorly understood.

Here, we report that activation of DCs by microbial agonists of TLR2 or TLR4, although leading to comparable activation of NF-$\kappa$B and MAPK family members, resulted in striking differences in cytokine and chemokine gene transcription, suggesting that signals emanating from different TLRs are not equivalent and that these pattern recognition receptors may differentially contribute to the polarization of adaptive immunity. A TLR4 agonist specifically promoted the production of the cytokine IL-12 p70, the chemokine IP-10, and the transcript for IFN-$\gamma$. Conversely, TLR2 stimulation resulted in preferential expression of IL-12p40.

Among these factors, IL-12 and IP-10 appear to be particularly interesting for their ability to contribute to T helper cell polarization. Due to its ability to stimulate IFN-$\gamma$ production in T cells, IL-12 is the key cytokine in directing the development of Th1 cells (26). It is believed that in its absence, T cell development proceeds by default toward the Th2 lineage. Bioactive IL-12 is produced by activated APC as a heterodimer composed of the p40 and p35 subunits. The p40 subunit is expressed in large excess of the p35 subunit, which is the limiting factor in controlling IL-12 p70 production (27, 28). IL-12 p40 can also form homodimers that have been shown to act as IL-12 receptor antagonist (24). It is therefore particularly interesting that TLR2 stimulation, although it fails to induce IL-12 p70 release due to defective p35 gene induction, is nevertheless still able to stimulate release of the inhibitory p40 homodimer, thus producing conditions that are predicted to favor Th2 lineage commitment. On the other hand, TLR2 stimulation preferentially induces p19 gene transcription. p19 is a recently identified protein that can form heterodimers with p40, creating a novel cytokine, IL-23, with activities similar to as well as different from IL-12. For example, compared with IL-12, IL-23 has a diminished ability to induce IFN-$\gamma$ secretion from activated T cells (25). It is also unclear which role IL-23 plays in T cells polarization. Due to the uncertainties regarding IL-23 biological activities, it is impossible at present to argue whether the lack of IL-12 production by TLR2-stimulated DCs can be compensated for by the higher p19 gene transcription in these cells.

Differential induction of IL-12 by TLR2 and TLR4 agonists has recently been reported in mouse macrophages (29). It is interesting to note that in that study, the lack of IL-12 production by TLR2 stimulation was due to absence of p40 transcripts.
rather than to a failure to transcribe p35, as in our study. Whether this reflects differences between human and mouse in the regulation of IL-12 biosynthesis is not clear.

Our finding that TLR4, but not TLR2, agonists stimulate production of IP-10 is also remarkable. IP-10 is a CXCL10 chemokine produced by different cell types in response to IFN-γ and microbial products. IP-10 is a chemoattractant for monocytes, NK cells (30, 31), and, importantly, TH1 cells, which have been shown to preferentially express the IP-10 receptor CXCR3 (32, 33). The inability of TLR2-stimulated DCs to produce detectable amounts of IP-10, in contrast to the high level obtained by TLR4 stimulation, may result in a defective recruitment of TH1 cells and is a further indication that signaling emanating from these receptors can differentially contribute to the polarization of the adaptive immune response.

Other factors were found to be differentially induced in our study. For example, the chemokine IL-8, a chemoattractant for neutrophils, was produced in much greater amounts by cells stimulated with TLR2 agonists than by cells stimulated with TLR4 agonists; conversely, the IFN-β gene was preferentially induced by TLR4 stimulation.

The use of pharmacological inhibitors of p38 MAPK and PI3K allowed us to establish the involvement of these kinases in the induction of several cytokine and chemokine genes in response to both TLR2 and TLR4 agonists. Interestingly, the transcripts of certain cytokines and chemokines, such as IL-12 p40, IL-10, and IP-10, appeared to be more affected by these inhibitors than others. Although our data cannot indicate whether either of these kinases is the TLR4-specific signaling molecule responsible for the differences between TLR2 and TLR4 stimulation, the involvement of PI3K in the induction of several cytokine and chemokine genes in response to both TLR2 and TLR4 agonists; conversely, the IFN-β gene was preferentially induced by TLR4 stimulation.

In conclusion, we have provided evidence that TLRs can activate different genes in response to different TLR agonists. This finding may have implications for the understanding of the role of TLRs in innate immunity and inflammation. Further studies are needed to elucidate the mechanisms by which TLRs differentially activate gene expression.
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