Human TLR10 Is a Functional Receptor, Expressed by B Cells and Plasmacytoid Dendritic Cells, Which Activates Gene Transcription through MyD88


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Human TLR10 is an orphan member of the TLR family. Genomic studies indicate that TLR10 is in a locus that also contains TLR1 and TLR6, two receptors known to function as coreceptors for TLR2. We have shown that TLR10 was not only able to homodimerize but also heterodimerized with TLRs 1 and 2. In addition, unlike TLR1 and TLR6, TLR10 was expressed in a highly restricted fashion as a highly N-glycosylated protein, which we detected in B cell lines, B cells from peripheral blood, and plasmacytoid dendritic cells from tonsil. We were also able to detect TLR10 in a CD1a+ DC subset derived from CD34+ progenitor cells which resemble Langerhans cells in the epidermis. Although we were unable to identify a specific ligand for TLR10, by using a recombinant CD4TLR10 molecule we also demonstrated that TLR10 directly associates with MyD88, the common Toll IL-1 receptor domain adapter. Additionally, we have characterized regions in the Toll IL-1 receptor domain of TLR10 that are essential in the activation of promoters from certain inflammatory cytokines. Even though TLR10 expression has not been detected in mice, we have identified a partial genomic sequence of the TLR10 gene that was present but nonfunctional and disrupted by a retroviral insertion in all mouse strains tested. However, a complete TLR10 sequence could be detected in the rat genome, indicating that a functional copy may be preserved in this species. The Journal of Immunology, 2005, 174: 2942–2950.

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significance of this is unknown, but may open the possibility to study this gene in an immunological model.

**Materials and Methods**

**Cell culture**

HEK293, 293T and T98G cell lines (American Type Culture Collection) were maintained in DMEM supplemented with 10% FBS, 2 mM l-glutamine, 100 U/ml penicillin G, and 100 µg/ml streptomycin (Invitrogen Life Technologies). B cell lines were obtained from American Type Culture Collection and were cultured in RPMI 1640 supplemented with 10% FBS and 0.5% 2-ME (Sigma-Aldrich), except for B lineage acute lymphoblastic leukemia cell lines pre-ALP and Dunatis which were produced and cultured in our laboratory as described previously (9–11). All cells were cultured at 37°C with 5% CO2.

**Cell preparations**

Umbilical cord blood samples, peripheral blood samples, and whole tonsils were obtained according to institutional guidelines. PBMCs were purified from human peripheral blood by Ficoll-Hypaque centrifugation. Monocytes were purified from PBMC by centrifugation over a 50% Percoll gradient followed by immunomagnetic depletion of T, B, and NK cells (12). CD34+ hemopoietic cells and monocytes were isolated from umbilical cord; both populations of cells were used to generate dendritic cells (DCs) and activated as previously described (12–14). Activation of DCs was performed by culturing overirradiated CD40L-expressing L cells in the presence or absence of IL-3 (15). For Western blot analysis, peripheral blood B cells were enriched from whole blood using CD19-coupled magnetic beads (Miltenyi Biotec). PDCs were purified from tonsils as previously described (16) and activated overnight in the presence of IL-3 with or without CD40L-expressing L cells (15).

**RT-PCR of human (h) TLR10**

Total cellular RNA was isolated using the GdSCN/CsCl gradient procedure (12). Cells were lysed in 4 M guanidine thiocyanate solution and the total RNA was isolated by centrifugation through a 5.7 M CsCl gradient. RNA was treated with DNase I before mRNA purification using the Oligotex-dT kit (Qiagen). Poly(A)+ RNA (2 µg) was used to make first-strand cDNA (Superscript kit; Invitrogen Life Technologies). First-strand cDNAs were prepared after DNase I treatment of 5 µg of total RNA using oligo(dT) primers (Pharmacia) using the Superscript kit. Synthesis of cDNAs was controlled by performing RT-PCR using β-actin primers. RT-PCR was performed using the following primers specific for a 670-bp fragment of the TLR10 cDNA: 5’-GATGCCGAGATGTCAGATTC-3’ (forward primer) and 5’-AAGCCCA CATTACGCTTAC-3’ (reverse primer).

Template DNA was added at 1 ng/µl and amplification was performed using the AmpliTaq enzyme and buffer (PerkinElmer), dNTPs at 0.8 mM, and DMSO at a 5% final concentration. Cycle conditions were 94°C for 1 min 60°C for 2 min, and 72°C for 3 min for 35 cycles.

**Northern blot analyses**

Human adult and fetal commercial tissue blots were used (MTN blots I–IV; Clontech Laboratories). Hybridization of Northern blots was performed with the 670-bp DNA fragment generated by RT-PCR labeled with [32P]dCTP (Amersham) using the High Prime kit (Boehringer Mannheim). Hybridization was performed overnight in rapid hybridization buffer at 60°C. High stringency washes were at 0.2× SSC and 0.2% SDS for two times for 30 min. Revelation of bands was performed after 15 days exposition on Biomax MR film (Kodak).

**Identification of TLR10 rodent homologues**

Potential rodent homologues of TLR10 were identified by the bioinformatics search of the partial rat and mouse genomes using tBLASTn (17). Sequences corresponding to rat and mouse TLR genes that had close homology to human TLR10, including areas specific to TLR10 and not seen in TLR1 or TLR6, were observed. These sequences were completed by amplification from the corresponding genomic DNA, cloning into pCRII-TOPO (Invitrogen Life Technologies), and sequencing using RP and –21 primers. When sequences differed from the public databases, several individual clones were sequenced and the difference was considered a polymorphism if present in at least two separate PCR or in all of the clones.
from a given reaction. Genomic DNA from two rat strains, Sprague Dawley and Lewis, was used as was DNA from nine unrelated mouse strains. Tissue samples (tail biopsies) of C57BL/6 and 129sv mice were obtained from Charles River Breeding Laboratories and DNA was extracted using the Qiagen DNeasy genomic DNA extraction kit (Qiagen). Genomic DNA from inbred mouse strains FVB/NJ, NZB, PL/J, NOD/Lt (Mus musculus), wild inbred strains Cast/Ei (Mus musculus castaneus), and SPRET/Ei (Mus spreus) was obtained from The Jackson Laboratory. Genomic DNA from Swiss mice was obtained from Promega. Amplification from the 5′ end of the mouse TLR10 coding sequence using the primers 5′-GATGTCACAAGAAGCCTGGGC (forward primer) and 5′-GACCATGACGACAGATTGTGTTG (reverse primer) gave bands of slightly different sizes from each strain of mouse. PCR amplification was performed for 35 cycles at 92°C for 1 min, 55 or 60°C for 1 min, and 72°C for 2 min. Identical results were achieved at amplification temperatures of 55 and 60°C. Sequences were cloned in pCRII-TOPO (Invitrogen Life Technologies) and sequenced.

Plasmid constructions

Flag-tagged hTLR2, hTLR4, and hemagglutinin (HA)-tagged hTLR3 were kind gifts from Prof. Alberto Mantovani (Università degli Studi di Milano, Milan, Italy). Other hTLRs and hMD-2 were amplified either from human genomic DNA or cDNA from PBMC, produced as above, and cloned into the pDISPLAY (Invitrogen Life Technologies) eukaryote expression vector which contains the Ig κ signal peptide and the NT-HA tag. We made use of a Ncol site downstream of the multiple cloning site to eliminate the c-myc epitope and the transmembrane domain. Alternative pDISPLAY vectors which encode c-myc or flag epitopes were produced in our laboratory by substitution of double-stranded oligonucleotides at the HA site of the vector. These were used to produce Flag-TRL10 and c-myc-MD-2. Constitutively active CD4TLR3, 4, and 10 were constructed by fusing cDNA encoding the extracellular domain of murine CD4 to the transmembrane and cytoplasmic domain of human TLR10, TLR4, and TLR3 (18).

Mutants were generated in the TIR domain of CD4TLR10 construct using the Gene Editor mutagenesis kit (Promega). Primers used to introduce mutations or deletions are as follows: 5′-CTACTTTGGACATTCGAAAAGC (P674H); 5′-CTTACCTGGACTCTGATAATG (KS688 – 689NR); 5′-GACCATCAGCCAAGTT (P674H); 5′-CTACTTTGACCATGGCAAAAGC (L780stop). Mutants were generated in the TIR domain of CD4TLR10 construct using the Gene Editor mutagenesis kit (Promega). Primers used to introduce mutations or deletions are as follows: 5′-CTACTTTGGACATTCGAAAAGC (P674H); 5′-CTTACCTGGACTCTGATAATG (KS688 – 689NR); 5′-GACCATCAGCCAAGTT (P674H); 5′-CTACTTTGACCATGGCAAAAGC (L780stop).

FIGURE 2. TLR10 is an N-glycosylated protein. HA-tagged TLR10 or MD-2 was transfected into HEK293T cells and 48 h after transfection cells were harvested, washed, and lysed. Lysates were treated with N-glycosidase F, O-glycosidase, and O-glycosidase plus neuraminidase for 16 h at 37°C.

Analysis of glycosylation of transiently transfected TLR10 and MD-2

For N-glycosylation analysis, 48 h after transfection the cells were lysed in 0.1 M phosphate (pH 7), 0.05% (w/v) SDS, 1% β-ME, 1% Igepal, and 50 mM EDTA (Nonidet P-40 buffer) and the lysates were treated with N-glycosidase F at 40 U/ml, O-glycosidase at 40 U/ml, and O-glycosidase plus neuraminidase at 40 U/ml for 16 h at 37°C.

Western blotting and immunoprecipitations of HEK293T cells

HEK293T cells were seeded into six-well plates and the following day 500 ng of the respective construct was transfected using FuGENE (Roche). Twenty-four hours after transfection for CD4TLRs and 48 h for full-length TLRs, cells were lysed in mild lysis buffer (MLB) containing 50 mM Tris-HCl (pH 8.0), 150 mM NaCl, 1% Triton X-100, 1 mM DTT, 0.5 mM PMSF, 1% aprotinin, 20 mM NaF, and 0.5 mM Sodium Orthovanadate.

Western blot analysis of purified B cells from blood, tonsil, and B cell lines

Cells were lysed in radioimmunoprecipitation assay buffer containing 50 mM Tris-HCl (pH 7.5) 150 mM NaCl, 1% Nonidet, 0.5% sodium deoxycholate, and 0.1% SDS. Protease inhibitors aprotinin, leupeptin, and PMSF (Roche) were added. For immunoprecipitations, cells were lysed in MLB and 40 μg of total protein was immunoprecipitated with 1.5 μg of the respective Ab for 2 h or overnight at 4°C in the presence of protein G-Sepharose. Beads were washed four times in MLB and 4% lithium dodecyl sulfate loading buffer was added. In general between 20 and 40 μg of total cellular protein (determined by Bradford assay; Bio-Rad) were used for SDS-NPAGE and immunoblotting (Invitrogen Life Technologies). After incubation with primary Abs, reactive proteins were detected with peroxidase-conjugated anti-mouse secondary Abs (Jackson ImmunoResearch Laboratories) and ECL (Amersham).

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Reporter assays

HEK293 cells were transiently transfected using FuGENE (Roche) with 100 ng of reporter construct along with 25 ng of CD4TLR expression vectors and, in certain experiments, in the presence or absence of 50 ng of MyD88DN into 24-well tissue culture plates with cells at 50% confluency. In addition, 1 ng of a construct directing expression of Renilla luciferase (under the control of the constitutively active CMV promoter) was used to normalize the transfection efficiency. Cells were harvested and analyzed for luciferase activity 24 h after transfection.

Luciferase assay

Cells were harvested and analyzed simultaneously for firefly and Renilla luciferase activity (to normalize) using the PerkinElmer Firelite reporter assay reagents.
Abs used

Human TLR10 clone 158C1114 (Imgenex), mCD4 (BD Pharmingen, France), M2 mouse anti-Flag (Sigma-Aldrich), mouse anti-HA (Roche), mouse anti-myc (clone 9E10; Roche), anti-CD3 (UCHT1; BD Pharmingen, France), and anti-CD28 (CLB-CD28/1; Sanquin).

Results

TLR10 is expressed by B cells and PDCs

Fig. 1A showed that DCs derived from monocytes in the presence of IL-4 and GM-CSF did not express TLR10. CD34/H11001-derived DCs expressed TLR10 during the early stages of differentiation but this disappeared upon culture with CD40L-expressing L cells. Culture of CD34/H11001 progenitor cells in GM-CSF and TNF gives rise to two populations of cells. The CD14/H11001 subset has been described as being similar to dermal DCs or circulating DCs, whereas the CD1a/H11001 subset resembles Langerhans cells in the epidermis (14). Interestingly, TLR10 expression was almost exclusively restricted to the CD1a/H11001 subset. As described by Kadowaki et al. (19), we also confirmed expression of TLR10 on PDCs (Fig. 1A, lower panel). We observed that the expression of TLR10 on PDCs isolated from tonsil remained relatively constant after maturation with IL-3 and CD40L. We were able to detect TLR10 expression in immunological tissue. An mRNA of 4.4 kb was detected by Northern blot analysis (Fig. 1B).

FIGURE 4. TLR10 interacts directly with MyD88: cotransfection of CD4TLR constructions with DNMyD88 and luciferase reporters NF-κB (A) or ENA-78 (B). HEK293 cells were harvested 24 h after transfection. Three independent experiments were performed, results generally deviated by <10% of the mean value. HEK293T cells were transfected with control and CD4TLR constructs in the presence or absence of MYD88NTFlag and harvested 24 h after transfection. C, Western blot control of MyD88NTFlag expression. D, Immunoprecipitation control of CD4TLR using the CD4 Ab. E, Immunoprecipitation using CD4 Ab, immunoprecipitated complexes were revealed with the Flag tag of MyD88NTFlag. 293T cells transfected only with MyD88NTFlag was used as a control for the Flag Ab.

FIGURE 5. A, CD4TLR10 mutants vs gene reporter activity: Alignments of the TIR domains of TLR1, TLR2, TLR6, and TLR10 shows the mutations and deletions generated in the TIR domain of TLR10. Sites of mutations are highlighted in gray. B, Figure representing the mutations and deletions generated in the TIR domain of TLR10. In dark gray are the areas representing the extracellular domain of murine CD4. Shown in light gray is the transmembrane domain and TIR domain of human TLR10. Sites of mutations are shown in black. The L and Ystop mutants were made in the carboxy-terminal where stop codons were introduced at amino acid positions 779 and 753, respectively. CD4TLR10 mutants vs luciferase reporters. Cotransfection of CD4TLR10 plasmids with NF-κB (C), IL-4 (D), and ENA-78 (E) reporters into HEK293 cells. Changes in luciferase activity were measured 24 h after transfection. Three independent experiments were performed; results generally deviated by <10% of the mean value.
We created a HA-tagged version of hTLR10; this construct was expressed in HEK293T cells which gave reproducible intracellular expression (data not shown). Western blotting of cell lysates expressed in HEK293T cells that were treated with TLR10, TLR6, and TLR1 showed a single protein of approximately 14 kDa. Coimmunoprecipitation studies (Fig. 3) revealed the association of TLR10 with itself as well as TLR2 and TLR1; we were unable however to observe a consistent association with TLR6 in two of the three experiments performed. TLR2 has already been shown to associate with TLR1 or TLR6 via the extracellular domains of these receptors (23–26). The fact that TLR10 associated with itself revealed a capacity to form homodimers.

MD-2 is an accessory molecule that associates with TLR4 (27). The coexpression and association of TLR4/MD-2 has been shown to be essential for the recognition of LPS by TLR4 (28). Coimmunoprecipitation experiments (Fig. 3B) showed that TLR4 associated with MD-2, but that TLR3 and TLR10 do not.

**TLR10 activates immune system promoters**

To evaluate the basic signaling potential of the TLR10 TIR domain, we generated a constitutively active construct by fusing the mouse CD4 extracellular portion with the TLR10 transmembrane and TIR domain. Previous experiments have shown that CD4TLR10 was able to activate NF-κB, ENA-78, and other gene promoters (18). In this study, we show for the first time that the signaling activity of TLR10 requires the adapter MyD88 (Fig. 4, A and B) but not TIRAP, TRAM, or TRIF (data not shown, using DN MyD88 and ENA-78 promoters). Furthermore, coimmunoprecipitation studies were performed to determine whether the TIR domain of TLR10 interacts directly with MyD88. We observed that DNMyD88 entirely blocked the signaling ability of CD4TLR10, partially CD4TLR4, but not CD4TLR3 to induce NF-κB and ENA-78 promoters. Furthermore, coimmunoprecipitation studies were performed to determine whether the TIR domain of TLR10 interacts directly with MyD88. These experiments show that CD4TLR10 interacted with MyD88 and with the control CD4TLR4 (Fig. 4, C–E), indicating for the first time MyD88 as the adapter molecule involved in the signalization of TLR10.
Mutations in the TLR10 TIR domain alters its signaling potential

The TIR domain is essential for signaling and TLR10 also contains the classical regions of particular signaling importance. Most TIR sequences have a conserved proline, which when mutated to histidine renders the protein unable to signal, probably due to an inability to associate with MyD88. Interestingly TLR3, the only TLR which does not associate with MyD88 has alanine at this position. Fig. 5A shows the positions where the mutants were made. Additionally, we noted a conserved potential serine protein kinase C phosphorylation site at position 692 in the TLR10 sequence. This sequence is conserved in all of the TIR domains. The sequence was mutated from KSYK to NRYK to remove the serine residue. We also made truncation mutations to remove parts of the nonconserved carboxy-terminal of the TLR10 TIR domain (Lstop and Ystop). Figure 5B schematically represents where deletions and stop codons were made in the carboxyl-terminal to mutate or shorten the open reading frame of the TLR10. We next examined the CD4TLR10 mutants in their ability to activate the NF-κB, ENA-78, and IL-4 promoters. Transfection of HEK293 cells with CD4TLR10 alone induced all three reporters (Fig. 6, C–E). Mutation of the conserved proline decreased luciferase activity of all three promoters. Mutation of the putative phosphorylation site at S692 caused a complete drop in reporter activity for IL-4 and NF-κB. Deletions L and Y in the TIR domain reduced NF-κB activity but a greater decline was observed with ENA-78 and IL-4 reporters; however, the Y deletion suppressed reporter activity of all promoters. Comparable levels of CD4TLR10 and mutant expression in HEK293T cells was detected by Western blotting using a mouse anti-CD4 Ab (data not shown).

A homologue of TLR10 is present in rat, but not in mouse

We determined whether other species had homologues of TLR10. Genomic DNA from humans, mouse, and rat were digested with EcoRI, XbaI, BamHI, and NotI. Using the 670-bp hTLR10 probe, single bands were observed for the human DNA, but for the rat DNA, a single band was not observed.
other species. Attempts at cross-species PCR using 20-mer oligonucleotides chosen at overlapping sites in the N-terminal coding region of hTLR10 with little homology to TLR1 and TLR6 did not yield any specific amplification (data not shown). We then used bioinformatics analysis to search for sequences with homology to hTLR10 in the genomic databases. Coding sequences similar to the hTLR10 protein were detected in mouse and rat BAC clones. Since the rat genomic clone contained sequences from the 5’ and 3’ coding sequence, PCR amplification from genomic DNA from the rat allowed us to identify the entire coding exon of this gene. However, for the mouse sequence only partial sequences could be found. Comparison of the human, rat, and mouse amino acid sequences allowed us to conclude that these sequences represent the rodent homologues of TLR10 (Fig. 6A). Interestingly, we detected sequences very similar to TLR1 and TLR6 on the same genomic clones as TLR10, suggesting that in the mouse, rat, and human the TLR1, TLR6, and TLR10 genes are clustered. The human and rat TLR10 proteins each showed a putative signal peptide (Fig 6B, boxed), a transmembrane region (Fig. 6B, boxed), and a conserved cytosolic TIR domain. Human and rat TLR10 show, respectively, seven and six potential sites for N-glycosylation (circled), of which six are conserved between the two proteins (Fig. 6B). Analysis of the genomic DNA sequences revealed that the mouse TLR10 gene is a nonfunctional gene, with numerous gaps, insertions, and phase changes, and with the TIR domain replaced by a retrovirus-like sequence. Amplification of mouse genomic DNA from nine unrelated mouse strains using PCR primers designed to amplify sequences around the peptide signal (forward primer) and the putative transmembrane domain (reverse primer) gave single bands (data not shown). Sequencing of these bands revealed sequences very similar to that detected on the BAC clone (C57BL/6 strain), with differences in certain repeated regions. Notably the inbred wild strains, CAST/Ei and SPRET/Ei, showed a shorter sequence and contained fewer repeats with respect to the C57BL/6 mice. This suggests that the mouse TLR10 gene has been lost by retroviral insertion and amplification of repeat regions after the separation of the mouse and rat lineages.

**Discussion**

TLR10 is the only remaining orphan member among the human TLRs. Research on this receptor has been hindered by the absence of a rodent homologue, a step that has considerably aided the identification of ligands for most TLRs. In this study we aimed to advance the field by defining an immunological model and assess receptor functionality even in the absence of a ligand. We showed that TLR10 had no mouse homologue due to the interruption of the mouse thr10 gene by a retroviral sequence. We hypothesize that this interruption had most likely occurred early in the evolution of the mouse lineage as seen by the fact that inbred wild mouse strains such as *M. spreus* or *M. musculus castaneus* of diverse geographical origins have similar vestigial thr10 gene sequences to those of highly inbred laboratory strains of *M. musculus*. These sequences represent the only mouse thr10 gene, shown at least for the C57BL/6 genome, in which thr10 can be isolated at the same locus as the thr1 and thr6 genes and in the same arrangement as seen for the human and rat copies of the three genes. Our data imply that although mice do not have a functional TLR10, it is probable that the thr10 gene in rat is expressed and functional. Recently, Hubert et al. (29) have isolated and characterized PDCs from rat spleen and reported the low level expression of TLR10 in these cells. This manuscript does not show the entire sequence of the thr10 gene from the rat; however, the primers used and their data suggest that they isolated the same gene as discussed in this report. Although the genetic manipulation of rat models are not as advanced as in mice, we anticipate that this will serve as an immunological tool in identifying the ligand.

In humans, TLR7, TLR9, and TLR10 expression is limited to granulocytes (in particular eosinophils), germinal center B cells, and PDCs (8, 30, 31). These expression data may indicate a role for TLR10 in terms of biological function in inducing type I IFN from PDCs as in the case of TLR7 and 9 (32) or in the case of B cell activation, inducing proliferation and cytokine secretion as observed for TLR9 in the presence of CpG motifs (8). We have confirmed and extended this expression to DCs generated in vitro resembling Langerhans cells (33). However, in several ex vivo Langerhans cell skin samples, we were unable to detect the expression of TLR10 (data not shown). This may suggest that the expression of TLR10 is directed by conditions similar to those in our in vitro cultures. Detection of the endogenous TLR10 protein has not been previously reported. We extended the RT-PCR results shown by Bourke et al. (8) by examining TLR10 protein expression. TLR10 was detected in all samples except Reh-6, an early pre-B cell precursor. However, TLR10 mRNA was detected in all cell lines tested, suggesting that mRNA expression starts early in the B cell lineage, but translation of TLR10 is by cells that are committed to B cell differentiation.

We observed for the first time protein complexes with TLR10 as a homodimer and association with TLR1 or TLR 2. In addition, ImageQuant densitometry analysis, using immunoprecipitation controls as standards, reveals that homodimerization of TLR10 has a binding affinity of 100%, whereas for a TLR1/10 heterodimer 87% was observed and for a TLR1/2 complex 80%. Phylogenetic analysis indicates that among all human TLRs, TLR10 is closely related to TLR1 and TLR6. From the sequence homology, the most plausible hypothesis is that a common TLR1/6/10 ancestor duplicated to produce a TLR1/6 precursor and TLR10 (34). Extensive research on TLR2 has shown that the association with TLR1 and TLR6 is necessary for efficient ligand binding and the discrimination of triacyl and diacyl lipopeptides from bacteria (35). Potentially TLR10 may act as a coreceptor to these TLRs and therefore share the same family of ligands. However, the expression pattern of TLR10 in B cells and PDCs (19, 22) is confined to cells that do not appear to express TLR2. It is thus possible that TLR10 does not associate with TLR2 in vivo. Homo- and heterodimer formation by TLRs and associated non-TLR surface Ags increases the potential for ligand recognition and immune system modulation and contributes to the wide range of recognized PAMPs (28). In assessing the nature of the TLR10 agonist, we tested TLR10 in single and cotransfections with TLR1, TLR2, and TLR6 with a luciferase-driven NF-κB minimal promoter to determine whether the TLR10 ligand is related to PAMPs already determined for this family of proteins. However, we were unable to clearly demonstrate that bacterial-derived components activated TLR10 to induce NF-κB. We did not test extensively potential viral ligands in this system. As mentioned previously, TLR10 is clearly expressed on PDCs which produce high levels of type I IFN in response to viral infection and activation of TLR7 and 9 (36); therefore, we cannot exclude that a potential TLR10 ligand could be viral (37). It is noteworthy that PDCs and B cells isolated from tonsils in this study would have been exposed to a reservoir of infection before isolation since human tonsils can, for example, harbor EBV, respiratory syncytial virus (38), HIV (39), and other herpesviruses (40), as well as a range of bacterial pathogens (41). From these reports, we conclude that TLR10 expression in PDCs from tonsils may be enhanced because cells may already be in a state of pathogen activation. It is also probable that TLR10, which also has an extremely restricted expression profile, responds to molecules derived uniquely from very specific...
The authors have no financial conflict of interest.

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Disclosure
The authors have no financial conflict of interest.

References


2950 TLR10 ACTIVATES GENE PROMOTERS VIA MyD88