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CD14-dependent Lipopolysaccharide-induced β-Defensin-2 Expression in Human Tracheobronchial Epithelium*

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The induction of host antimicrobial molecules following binding of pathogen components to pattern recognition receptors such as CD14 and the Toll-like receptors (TLRs) is a key feature of innate immunity. The human airway epithelium is an important environmental interface, but LPS recognition pathways have not been determined. We hypothesized that LPS would trigger β−defensin (hBD2) mRNA in human tracheobronchial epithelial (hTBE) cells through a CD14-dependent mechanism, ultimately activating NF-κB. An average 3-fold increase in hBD2 mRNA occurs 24 h after LPS challenge of hTBE cells. For the first time, we demonstrate the presence of CD14 mRNA and cell surface protein in hTBE cells and show that CD14 neutralization abolishes LPS induction of hBD2 mRNA. Furthermore, we demonstrate TLR mRNA in hTBE cells and NF-κB activation following LPS. Thus, LPS induction of hBD2 in hTBE cells requires CD14, which may complex with a TLR to ultimately activate NF-κB.

The innate immune response comprises the first line of host defense against pathogenic microorganisms. Many protective elements including nitric oxide, peptides/proteins, and whole cells such as phagocytes and natural killer cells have been conserved during evolution. Studies in *Drosophila* illustrate the primary importance of pathogen recognition and induction of antimicrobial molecules. Toll mutations preventing antifungal peptide induction render mutant flies susceptible to fungal infection (1). Signaling through *Drosophila* Toll results in activation of members of the NF-κB family of transcription factors and expression of antimicrobial factors (2). Pathways for pattern recognition and signaling have been identified in mammals where Toll-like receptors (TLRs)[1] (3, 4) and NF-κB provide functions similar to *Drosophila* Toll and dorsal or Dif, respectively (5, 6).

Innate immunity is induced when loosely defined recognition elements of microbes bind to pattern recognition receptors present on both phagocytic and epithelial cells (7). These receptors include the mannose receptor (8), the GPI-linked LPS co-receptor CD14 (9) and the TLRs (3). In human macrophages, TLR2 acts in concert with CD14 to bind LPS and initiate a signaling cascade (10, 11). The mouse TLR4 homologue is an important determinant of LPS responsiveness (12). In general, activation of pattern recognition receptors induces host defense gene products.

As a primary interface between pathogens and the environment, epithelial cells lining the mammalian airways are a crucial site for the innate immune response. It has been proposed that dysfunction of innate immunity may result in recurrent airway infections as seen in cystic fibrosis (13). The bovine β-defensin tracheal antimicrobial peptide (TAP) serves as a paradigm for induction of innate immunity in the airway (14). The TAP gene is expressed in the ciliated airway epithelium (15) and is induced following experimental bacterial infection (14). *In vitro* incubation of bovine tracheal epithelial cells with LPS increases TAP mRNA levels via a CD14-mediated response (14), culminating in NF-κB activation and transcriptional up-regulation of the TAP gene (16). Thus, in the bovine airway epithelium antimicrobial peptides are induced through a well defined recognition and activation pathway that helps prevent microbial colonization.

Two β-defensins are present in human epithelia. Human β-defensin-1 (hBD1) (17) is highly expressed in urogenital tissues (18), and to a lesser extent in airway and other epithelia, entirely in a constitutive manner (19). Human β-defensin-2 (hBD2) was initially found in psoriatic skin and is present in cultured keratinocytes in response to bacteria (20). The hBD2 gene is similar to TAP and includes three NF-κB consensus sequences upstream from the transcriptional initiation site (21). The mRNA for hBD2 is present in human lung (22) and is up-regulated in chronic inflammation and by the proinflammatory mediator IL-1β (23). Thus, hBD2 is a host defense molecule whose production is induced in response to infection and inflammation. Purified hBD2 peptide acts synergistically with other antibacterial components of the airway surface fluid, including lysozyme and lactoferrin (22), suggesting a role in maintaining a pathogen-free environment.

As an innate immune response tissue, the human airway must recognize pathogen-associated molecular patterns such as LPS via cell surface receptors. Surprisingly, neither CD14 nor TLRs have been documented in human tracheobronchial epithelial (hTBE) cells. We hypothesized that LPS would trigger hBD2 expression possibly through a CD14-mediated recognition, ultimately resulting in hBD2 induction via activation of NF-κB. To establish this paradigm, we analyzed the hBD2 mRNA response to LPS in hTBE cells and determined the role of CD14. Furthermore, we document expression of TLRs in

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[1] The abbreviations used are: TLR, Toll-like receptor; hTLR, human TLR; LPS, lipopolysaccharide; TAP, tracheal antimicrobial peptide; hBD1 and -2, human β−defensin 1 and 2, respectively; hTBE cell, human tracheobronchial epithelial cell; IL, interleukin; PCR, polymerase chain reaction; RT-PCR, reverse transcription-PCR; GPI, glycosylphosphatidylinositol.

TABLE I

Gene (accession no.)	Forward primer (5′ → 3′)	Bases	Reverse primer (5′ → 3′)	Bases
γ-Actin (M19283)	GCCAACAGAGAGAAGATGAC	1350–1369	AGGAAGGAAGGCTGGAAC	2087–2070
TLR1 (U88540)	TGCCCTGCCTATATGCAA	381–398	GAACACATCGCTGACAACT	936–918
TLR2 (U88878)	CCTACATTAGCAACAGTGACCTAC	323–346	ATCTCGCAGTTCCAAACATTCCA	822–800
TLR3 (U88879)	CGCCAACTTCACAAGGTA	277–294	GGAAGCCAAGCAAAGGAA	966–949
TLR4 (U88880)	AGATGGGGCATATCAGAGC	446–464	CCAGAACCAAACGATGGAC	945–927
TLR5 (U88881)	TTCTGACTGCATTAAGGGGAC	93–113	TTGAGCAAAGCATTCTGCAC	660–641
TLR6 (AB020807)	CCTCAACCACATAGAAACGAC	832–852	CACCACTATACTCTCAACCCAA	1363–1342

these cells and demonstrate activation of NF-κB in response to LPS.

EXPERIMENTAL PROCEDURES

Reagents—Escherichia coli LPS serotype O127:B8, Igepal CA-630, nitro blue tetrazolium, and 5-bromo-4-chloro-3-indolylphosphate were from Sigma. Mouse monoclonal My4 and mouse IgG2b antibodies were obtained from Beckman Coulter (Miami, FL). IL-1β was from R&D Systems (Minneapolis, MN).

Primary Culture of hTBE Cells—hTBE cells were isolated under the auspices of Institutional Review Board approved protocols as described in detail previously (24). Passage 1 or 2 hTBE cells were cultured at an air-liquid interface on 24-mm T-COL membrane supports (Costar, Cambridge, MA) for the number of days indicated in each experiment. Initial seeding density was $0.7–1 \times 10^6$ cells/support. Growth medium was modified from that in Ref. 24 by the use of bovine pituitary extract from Upstate Biotechnology, Inc. (Lake Placid, NY) and the elimination of antibiotics. The medium was periodically tested for endotoxin levels with the Limulus Amebocyte Lysate assay (BioWhittaker (Walkersville, MD) or Associates of Cape Cod (Falmouth, MA)), and endotoxin levels were below 100 pg/ml.

LPS and Inflammatory Stimulus—LPS (*E. coli*) at 5 μg/ml was added either apically or basolaterally to cultures with 5% human serum (Sigma catalog no. H4522) as a source of LPS-binding protein. It is important to note that our cells are normally cultured at an air-liquid interface and that the cells respond to apical flooding by increased acid production (yellowing of the media). In preliminary experiments, we challenged cells with LPS from both sides simultaneously or from only the basal or apical side (with apical flooding controls). The most consistent response was observed with a basal challenge while maintaining an air-liquid interface. Since we observed CD14 on both the apical and basal membrane (this study) and to avoid disturbance of the air-liquid interface, we chose basal challenge for our studies. IL-1β was added basolaterally at 25 ng/ml. For blocking experiments, My4 or IgG2b was added to the cultures both apically and basolaterally 20 min prior to the addition of LPS.

Northern Blot Analysis—RNA was isolated with TRI-reagent according to the manufacturer's protocol (MRC, Cincinnati, OH). Unless otherwise noted, all of the RNA from one 24-mm T-COL was electrophoresed in a single lane on 1.2% agarose-formaldehyde gels. Northern blot analysis was carried out by standard capillary transfer to a Hybond N membrane (Amersham Pharmacia Biotech). Blots were hybridized with Quikhyb (Stratagene, La Jolla, CA) with 2×10^6 counts per ml of ^{32}P-labeled probe. For hBD2, either a random primed fragment (bp11–265; accession number Z71389) or an end-labeled oligonucleotide (5′-ATATGAAGAGGAACGAGAAGAGGAGATACAAGACCCTCAT-3′) was used. Fragments for CD14 (base pairs 52–563, accession number X06882) and TLR4 (base pairs 446–945, accession number U88880) were PCR-cloned with the Invitrogen (Carlsbad, CA) TOPO TA or TA kit, respectively. The inserts were isolated and random prime-labeled (Rediprime; Amersham Pharmacia Biotech) for use as probes. Inserts for TLR2 (base pairs 2012–2600; accession number U88878) and γ-actin (*Xho*I fragment of pHFγA-1 (25) encompassing the entire cDNA sequence) also were random primed. All accession numbers refer to GenBank™. Quantitation was performed with a Molecular Dynamics PhosphorImager.

IL-8 Production—The production of IL-8 was measured by enzyme-linked immunosorbent assay (R&D Systems) in samples of the growth medium taken before and after LPS challenge.

Reverse Transcription (RT)-PCR—RT-PCR was performed as described previously (24). Seven-day-old cultures were used for the RNA isolation. Primers used are listed in Table I. The THP-1 cells were obtained from the ATCC (TIB 202) and were maintained in RPMI 1640 with 5×10^{-5} M 2-mercaptoethanol plus 10% fetal bovine serum. Lung RNA was obtained from CLONTECH (Palo Alto, CA).

Western Blot and Immunoprecipitation Analysis—For detection of hBD2, Western blots were performed as in Ref. 18. Briefly, cells were lysed in 5% acetic acid, and acid-soluble proteins were extracted overnight at 4 °C. After lyophilization, proteins were separated on acid urea-polyacrylamide gels and electroblotted to an Immobilon P^{SQ} membrane (Millipore Corp., Bedford, MA). Membranes were blocked and incubated with a 1:1000 dilution of anti-hBD2 (the kind gift of Tomas Ganz, UCLA, Los Angeles, CA) or normal rabbit serum overnight. Subsequently, blots were incubated with an alkaline phosphatase-conjugated secondary antibody (Jackson Immunoresearch, West Grove, PA) and visualized with nitro blue tetrazolium/5-bromo-4-chloro-3-indolylphosphate.

Cell surface biotinylation, immunoprecipitation, and Western blots were performed as described (26). Briefly, 7-day-old cultures of hTBE cells were surface-biotinylated with EZ-Link sulfo-NHS-biotin (Pierce) according to the manufacturer's protocol. Proteins were isolated in radioimmune precipitation buffer and immunoprecipitated with the My4 antibody or an IgG2b isotype control. Immunoprecipitated proteins were run on a 4–20% acrylamide gel (NOVEX, San Diego, CA) and electroblotted to a polyvinylidene difluoride membrane. Biotinylated proteins were detected by streptavidin-linked horseradish peroxidase and chemiluminescence (Pierce).

Electrophoretic Mobility Shift Assays—Cells were harvested from the membrane in phosphate-buffered saline plus protease inhibitors (1 mM phenylmethylsulfonyl fluoride, 1.2 μg/ml leupeptin, 1 μg/ml pepstatin, 0.5 mM EDTA) with a cell scraper. Extracts were prepared according to the method of Dignam (27) with the modification that Igepal CA-630 (0.25%) was added to buffer A before homogenization, and buffer D was added directly to the cleared supernatant rather than dialyzed against buffer D. Complementary oligonucleotides containing the NF-κB consensus sequence from the class I MHC promoter (28) were annealed and labeled by end filling with Klenow and [^{32}P]dCTP. Binding reactions were performed according to Ref. 29, and complexes were separated by 5% nondenaturing polyacrylamide gel electrophoresis in Tris-glycine-EDTA buffer. For competition experiments, 20 μg of NF-κB consensus or mutant oligonucleotides from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA) were added to the binding reaction.

RESULTS

Inducibility of antimicrobial factors by bacterial products and/or inflammatory mediators is a key feature of innate immunity. LPS induction of hBD2 gene expression was examined in passage 1 or 2 hTBE grown in culture at an air-liquid interface. Cells were grown for 7, 14, or 21 days, which corresponds to distinct stages of mucociliary differentiation (24), and were treated with *E. coli* LPS at 1 or 5 μg/ml in the presence of human serum as a source of LPS-binding protein. Fig. 1 shows that hBD2 mRNA levels are highest early in culture and that expression is induced after incubation with LPS. In subsequent experiments conducted at 7 days postseeding, steady-state levels of hBD2 mRNA increased an average of 3-fold following LPS (range 1.3–6.3-fold, 10 separate experiments, with seven different patient cell samples) and up to 16-fold following IL-1β (data not shown), which is consistent with published results (23, 30).

The LPS dose-response relationship for hBD2 induction in hTBE cells is shown in Fig. 2*A*, which is a graphical representation of the results from a Northern blot with triplicate samples. An LPS dose of 10 ng/ml, which typically induces strong responses in monocyte-derived cells, did not alter hBD2 expression in hTBE cells, but hBD2 mRNA increased following 100 ng/ml to 1 μg/ml of LPS. As shown in Fig. 2*B*, we examined LPS stimulation of the proinflammatory cytokine IL-8 in the same cultures. The basal expression of IL-8 is approximately 10-fold

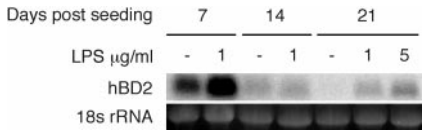

FIG. 1. **Induction of hBD2 mRNA in hTBE cells by LPS.** Northern blot analysis of mRNA from hTBE cells grown for 7, 14, or 21 days. Cells were induced with LPS (1 or 5 μg/ml), and RNA was harvested 24 h later. The blot was hybridized with a probe for hBD2, and the ethidium bromide-stained 18 S rRNA band is shown as a loading control.

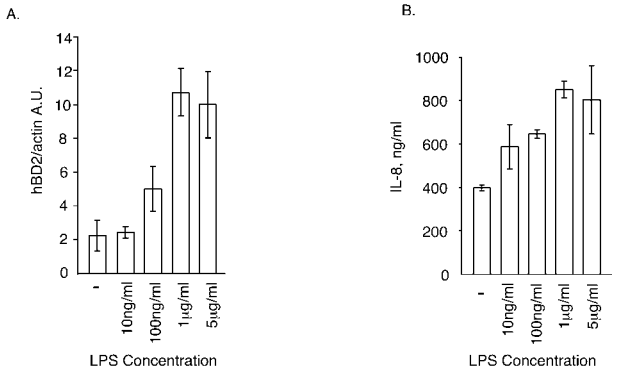

FIG. 2. **Steady state hBD2 mRNA and production of IL-8 by hTBE cells in response to LPS.** *A*, cells were cultured for 7 days and exposed to varying amounts of *E. coli* LPS. RNA was harvested 24 h later, and the resulting Northern blot was hybridized with probes for hBD2 and subsequently for γ-actin. The hBD2 signal was normalized to γ-actin and is presented in arbitrary units (*A.U.*). *B*, enzyme-linked immunosorbent assay measurements of IL-8 protein in the basolateral medium after treatment of cells with LPS as described above. In both *A* and *B*, the overall LPS effect is significant by analysis of variance ($p < 0.001$ for hBD2; $p = 0.007$ for IL-8). Both the 1- and 5-μg doses are significantly different from no LPS by the Tukey test for multiple comparisons ($p = 0.002$ and 0.003 for hBD2 and $p = 0.007$ and $p = 0.015$ for IL-8, respectively). Values are the mean ± S.E. of triplicate samples.

higher in the presence of serum than in cultures with media alone (data not shown). However, $\geq$1 μg/ml LPS induced a significant increase over this elevated base line.

The time course of hBD2 mRNA induction is shown in Fig. 3*A*. LPS increased hBD2 mRNA at 12 and 24 h, but not 6 h, following challenge. Basal hBD2 expression declined over 24 h, possibly in response to the serum added to the medium as a source of LPS-binding protein. Subsequent analyses were performed 24 h following LPS addition. Fig. 3*B* demonstrates a corresponding LPS-induced increase in hBD2 protein as detected by Western blot.

The GPI-linked cell surface protein, CD14, is known to participate in LPS responsiveness in bovine tracheal epithelial cells (14). We examined hTBE cells for the expression and production of CD14, as well as its involvement in the up-regulation of hBD2. Immunoprecipitation of biotinylated surface proteins reveals that membrane-bound CD14 is found on both the apical and basolateral surfaces of hTBE cells (Fig. 4*A*). The Northern blot shown in Fig. 4*B* indicates that mRNA for CD14 is present in hTBE cells, and the levels of CD14 are not altered in response to LPS. To test the role of CD14 in the stimulation of hBD2 gene expression, we challenged cultures with LPS in the presence of either My4, a neutralizing monoclonal antibody to CD14, or an IgG2b isotype control. The Northern blot in Fig. 4*B* and the corresponding graphical representation in Fig. 4*C* are representative of three experiments and show that My4 blocks the up-regulation of hBD2 by LPS. Thus, CD14, either membrane-bound or the soluble form, is necessary for the LPS-induced increase in hBD2 mRNA.

As a GPI-linked protein without a cytoplasmic domain, CD14 cannot act alone as a classical signal transduction molecule. As noted above, recent studies suggest that CD14-TLR complexes may function to initiate a signal transduction event. We examined hTBE cells for the expression of TLR genes by RT-PCR (Fig. 5*A*). Our results show that mRNA for all six published hTLRs is expressed in these cultures. Based on published results (10, 11, 31) TLRs 2 and 4 are probable LPS-signaling intermediates. Northern blot analysis shown in Fig. 5*B* indicates that hTLR2 is abundantly expressed in hTBE cells and that there is no induction by LPS. Human TLR4 expression is also detectable by Northern blot but is much less abundant than TLR2 and is also not regulated by LPS (Fig. 5*C*).

The promoter region of the hBD2 gene has three NF-κB consensus binding sites, suggesting a role for this family of transcription factors as a mechanism for gene regulation. Using electrophoretic mobility shift assays, we determined whether LPS-induced hBD2 expression was associated with activation of NF-κB. Upon induction with LPS for varying time periods, we isolated nuclear extracts from hTBE cells. THP-1 cells were used as a positive control (32). Fig. 6*A* indicates that NF-κB is activated after a 1-h incubation with LPS in hTBE cells and persists through 8 h. A stronger and more persistent shift is seen in response to IL-1β. Preincubation of the extracts with unlabeled NF-κB consensus oligonucleotide, but not with unlabeled mutant oligonucleotide, effectively competes for binding to the labeled oligonucleotide demonstrating specificity (Fig. 6*B*).

DISCUSSION

The airway epithelium comprises an important barrier against invasion by airborne pathogens. We show that epithelial cells lining the respiratory tract induce the host defense gene hBD2 at both the mRNA and protein level in response to LPS, a pathogen-associated pattern molecule. Human β-defensin-2 mRNA also is increased by IL-1β (23, 30), suggesting that a similar protective effect accompanies inflammation even when not directly induced by bacterial products. High levels of β-defensin gene expression have been observed at sites of inflammation in the bovine tongue (33) and airway (34). There are several examples of β-defensin induction in response to infection and inflammation by human tissues that provide barrier functions, including the oral mucosa (30, 35), occular epithelium (36), and intestinal epithelium (37). Together these data suggest that a peptide-based antimicrobial host response during infection and inflammation is intrinsic to mucosal surfaces.

The molecular pathway for the induction of antimicrobial peptides in the airway is both similar to and different from circulating professional phagocytes, such as monocytes. While initially discovered and extensively studied in myeloid-derived cells, CD14 is also expressed by bovine and murine epithelial cells (14, 38). Our results demonstrate both CD14 mRNA and cell surface protein in human airway epithelial cells. We found that CD14 mRNA was not increased in response to LPS, whereas it is in monocytes (39). The CD14-specific antibody, My4, inhibited LPS-induced hBD2 expression in hTBE cells, which suggests a critical role for CD14 in the mechanism by which airway epithelial cells recognize and respond to bacterial products.

Induction of hBD2 or IL-8 in hTBE requires relatively high concentrations (1–5 μg/ml) of LPS, whereas phagocytic cells or bovine epithelial cells are activated by 10–20 ng/ml concentrations. Thus, while CD14 is an important mediator of LPS responsiveness in hTBE cells, initiation and coupling to downstream signal transduction events appears to be much less efficient than in monocyte-derived cells. Primary cultures of bovine tracheal epithelial cells respond to lower levels of LPS

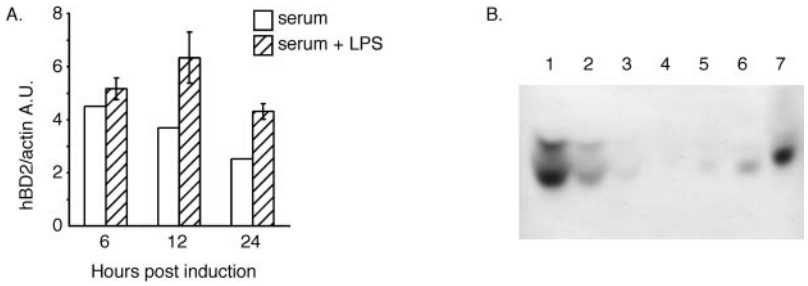

FIG. 3. **LPS induction of hBD2 mRNA and protein.** *A*, hTBE cells cultured for 7 days were treated with LPS (5 μg/ml) for 6, 12, or 24 h. RNA was isolated, and the resulting Northern blot was hybridized to hBD2 and γ-actin probes. *Open boxes* are the mean of duplicate samples, and the *hatched boxes* are the mean ± S.E. of triplicate values. Although LPS induction of hBD2 was relatively low in this experiment, the 24-h time point was significantly greater than control when compared by Student's *t* test with Bonferroni's multiple comparison correction. *p* values for 6, 12, and 24 h were 0.44, 0.08, and 0.01, respectively. *B*, acid urea-polyacrylamide gel electrophoresis Western blot analysis of hBD2 in acid-soluble proteins from hTBE cells. *Lanes 1–3* represent 10, 3, and 1 ng of hBD2 standard. Proteins in *lanes 4–7* were isolated from a single 24-mm hTBE culture exposed for 24 h to medium alone, medium plus serum, medium plus serum and LPS, or medium with IL-1β, respectively. No bands were visible on a duplicate blot treated with normal rabbit serum (not shown). *A.U.*, arbitrary units.

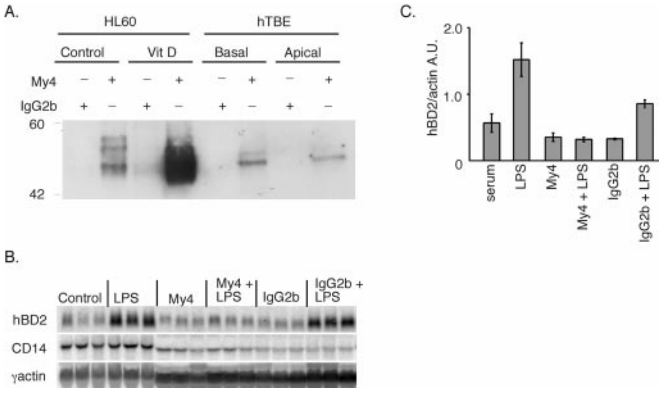

FIG. 4. **The role of CD14 in the LPS-mediated induction of hBD2 gene expression.** *A*, biotinylated surface proteins were immunoprecipitated with the My4 antibody specific to CD14 or an IgG2b isotype control. Electrophoresed immunoprecipitated proteins were blotted and then visualized by streptavidin-conjugated horseradish peroxidase and chemiluminescent detection. As a positive control, HL60 cells, a myeloid cell line, were differentiated with 50 nM vitamin D. *B*, My4, a neutralizing antibody to CD14, was added to 7-day-old hTBE cultures at 5 μg/ml both apically and basolaterally for 20 min prior to 24-h basolateral LPS stimulation. IgG2b was used as an isotype control. RNA was isolated from the cultures, and the resulting Northern blot was hybridized sequentially with probes for hBD2, CD14, and γ-actin. The blot shown is representative of three experiments. *C*, a graphical representation of the blot in *B*. hBD2 signals were standardized to γ-actin and are presented in arbitrary units. When analyzed by analysis of variance, there was a very significant overall treatment effect ($p < 0.001$). My4 abolished LPS induction of hBD2 mRNA, ($p < 0.001$ for LPS alone *versus* My4 plus LPS according to the Tukey test for multiple comparisons). The antibody control for My4 (IgG2b plus LPS) is significantly greater than its own control (IgG2b alone), $p < 0.05$. *A.U.*, arbitrary units.

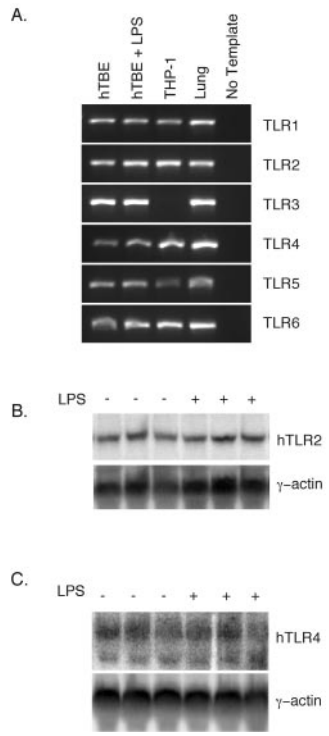

FIG. 5. **Expression of Toll-like receptors on hTBE cells.** *A*, RT-PCR analysis of hTLR1–6 with total mRNA from hTBE cells. The absence of genomic DNA was verified by an intron-spanning primer pair from the γ-actin gene. All bands are of the predicted size. RNA isolated from 7-day-old hTBE cultures with and without LPS induction was blotted and hybridized with either hTLR2 (*B*) or hTLR4 (*C*) and subsequently with γ-actin probes.

than passaged hTBE cells.[2] Surface CD14 in passage 1 hTBE cells was detected by immunoprecipitation, but expression was much less than in the vitamin D_3-differentiated HL60 cells used as a positive control. Further studies are necessary to determine CD14 protein levels in normal and diseased human airways *in vivo*.

The low sensitivity of the hTBE cells to LPS could be due to desensitization during culture. The medium for hTBE cells contains several biologicals including albumin, EGF, transferrin, and bovine pituitary extract. The practical limit of endotoxin reduction was 100 pg/ml. Thus, desensitization by basal endotoxin levels in the medium could have contributed to the apparent resistance of hTBE cells to LPS. However, polymyxin B addition did not reduce basal levels of IL-8 production (data not shown). As demonstrated by others, LPS desensitization of monocytic cell lines and murine macrophages appears to require 20–100 ng of LPS/ml (40–42). In LPS-tolerant cells, DNA binding activity in NF-κB gel shift assays consists mostly of p50 homodimers in LPS-tolerant cells (40, 41). The NF-κB gel shift assays in our studies are consistent with those seen in LPS-sensitive Mono Mac 6 cells, where the low mobility upper band representing the p50/p65 heterodimer is more prominent. We hypothesize that the low LPS responsiveness of hTBE cells reflects the low level of CD14 expression compared with monocytic cells. In support of this hypothesis are the findings that the response to LPS in THP-1 cells correlates inversely with the level of CD14 expression (43).

The lack of a cytoplasmic domain in GPI-anchored CD14 implies that it acts in concert with other proteins to transduce signals. Several lines of evidence suggest that TLRs contribute

[2] G. Diamond, unpublished observations.

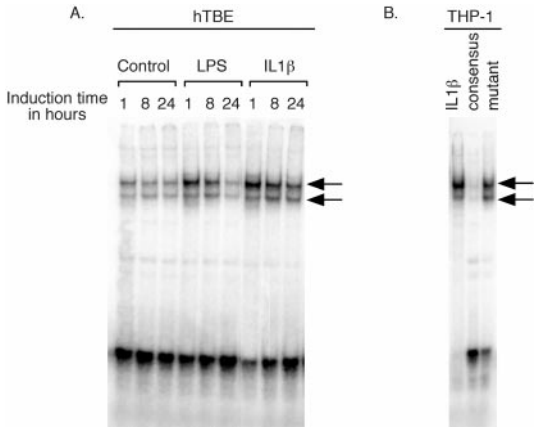

FIG. 6. **Activation of NF-κB in hTBE cells in response to LPS and inflammatory mediators.** *A*, nuclear extracts were isolated from hTBE cultures after incubation with LPS or IL-1β for 1, 8, or 24 h. The *arrows* indicate the shifted bands. *B*, the shifted bands were shown to be specific for NF-κB by competition with an oligonucleotide containing a consensus or mutant NF-κB site. The upper band indicated by the *arrow* contains the p65 subunit of NF-κB detectable by supershift (data not shown). Similar results were obtained with cells derived from two additional patient samples.

transmembrane signaling functions. Recent reports indicate that TLR2 mediates both LPS sensitivity (44) and responsiveness to Gram-positive bacteria (45) in transfected 293 cells. However, TLR2 null hamster macrophages still respond to LPS (46), suggesting a complementary function for other TLRs including TLR4. It is now known that mouse TLR4 is equivalent to the mouse LPS gene conferring LPS sensitivity (12). Using RT-PCR, we demonstrate mRNA for the six published hTLR sequences in hTBE cells and have established that hTLR2 and -4 are present at levels detectable by Northern blot of whole RNA. Further studies are needed to clearly elucidate the specific roles of TLR2 and/or -4 in initiating signal transduction responses in the airway. This will require the development of tools including neutralizing antibodies analogous to My4, successful strategies to generate dominant negative phenotypes in difficult to transfect polarized hTBE cells, and genetically engineered animals.

Studies of the *Drosophila* Toll pathway for antimicrobial factor induction and the discovery of inducible antimicrobial peptide expression in mammals suggest an evolutionarily conserved activation pathway. MUC2 mucin, which can be considered an innate immune molecule, increases in response to *Psuedomonas aeruginosa* through NF-κB activation (47). We demonstrate that hTBE cells activate the NF-κB pathway in response to LPS similarly to bovine epithelial cells (16). Interestingly, hTBE activation of NF-κB is of a lower magnitude and more transient following LPS compared with IL-1β, which is consistent with the typically lower -fold induction of hBD2 mRNA that we observed. It will be interesting to compare and contrast the function of components in the IL-1β and LPS signal transduction pathways to discover the basis for this difference.

In summary, the human airway epithelium responds to products from infectious microorganisms by inducing antimicrobial peptides. The activation pathway shares elements with cells of the myeloid lineage in that the epithelium expresses pathogen-associated recognition receptors. These receptors identify and transduce a signal ultimately activating NF-κB, which in turn up-regulates antimicrobial peptide genes. Taken together with studies in other organ systems, this innate immune mechanism is probably intrinsic to most if not all epithelia, providing a barrier function. Although *in vitro* studies such as ours introduce a level of uncertainty, it appears that hTBE cells require much higher doses of LPS than blood monocytes. This is perhaps not surprising in view of the potential for very high LPS aerosol exposure in certain environments (48). It will be important to determine the mechanisms, possibly involving CD14 and hTLRs, by which the organism balances defense against inhaled microbes with the potential pathophysiology inherent to chronic airway inflammation.

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