Pneumococci induced TLR- and Rac1-dependent NF-κB-recruitment to the IL-8 promoter in lung epithelial cells

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PNEUMOCOCCI ARE THE COMMONET ID OF DEATH DUE TO INFECTIOUS DISEASES IN INDUSTRIALIZED COUNTRIES (14). OVER 40% OF ALL CASES ARE DUE TO STREPTOCOCCUS PNEUMONIAE, WHICH IS THE MOST COMMON ETIOLOGIC AGENT OF COMMUNITY-ACQUIRED PNEUMONIA (5, 31). ACTIVATION OF LUNG EPITHELIAL CELLS BY PNEUMOCOCCI ENTEROGENICALLY BY NOD2, BY INVADING BACTERIA, AND BY INTRACELLULAR PATHWAYS (7). SIGNALS FROM THE CYTOSKELETON (36, 37) CAN BE MODULATED BY THE TNF-α RECEPTOR (19, 34, 40) AND APOPTOSIS REGULATION (18).

In this study we analyzed activation of and signal transduction in lung epithelial cells by pneumococci. We found that pneumococci are synergistically recognized by TLR1 and TLR2 dimers activated by triacylated lipoproteins, whereas TLR2/TLR6 recognized diacylated lipoproteins (49). Whether dimerization also plays a role in recognition of pneumococci is not known. In addition to identification of extracellular pneumococci by TLRs, stimulation of nucleotide-binding oligomerization domain proteins, particularly in NOD2, by invading pneumococci may also contribute significantly to host cell activation (32).
ylation of serine 276, whereas Rac1 did not regulate pneumococci-induced binding of phosphorylated c-Jun. Together, S. pneumoniae activated epithelial cells by TLR1/2 and PI3K-Rac1-NF-κB pathway.

**MATERIALS AND METHODS**

**Materials.** DMEM, fetal calf serum (FCS), trypsin-EDTA solution, CA-650, and antibiotics were obtained from Life Technologies (Karlsruhe, Germany). Nsc23766 (Nsc) was obtained from the Drug Synthesis and Chemistry Branch, Developmental Therapeutics Program, Division of Cancer Treatment and Diagnosis, National Cancer Institute, Bethesda, MD. Drs. R. R. Schumann and N. Schroeder (Charité—Universitätsmedizin Berlin, Germany) kindly supplied recombinant human LBP. All other chemicals used were of analytical grade and obtained from commercial sources.

**Cell lines.** Bronchial epithelial cell line BEAS-2B was a kind gift of Dr. Curtis Harris (National Institutes of Health, Bethesda, MD) (12, 35). Human embryonic kidney cells HEK-293 were purchased from ATCC (Rockville, MD). Chinese hamster ovarian cells (CHO-TLR4/CD14) stably expressing CD14, human (h) TLR4, and an NF-κB-dependent reporter gene (CD25) vector were kindly provided by Dr. Douglas Golenbock (University of Massachusetts Medical School, Worcester, MA) (17, 28).

**Bacterial strains and isogenic mutants.** S. pneumoniae R6x, the unencapsulated derivative of D39, was used in this study (11, 41, 42). S. pneumoniae were cultured in Todd-Hewitt broth (Oxoid, Basing-stoke, UK) supplemented with 0.5% yeast extract (THY) to mid-log phase or grown on blood agar.

**Pneumolysin.** Recombinant pneumolysin was expressed in Escherichia coli and was purified from cell extracts as described elsewhere (39). Protein homogeneity was confirmed by SDS-PAGE. The stock solution of pneumolysin was stored at −20°C and was reconstituted in PBS containing bacterial endotoxin. Our stock concentration was 0.21 mg/ml, which corresponds to 1.3 × 10^6 hemolytic units/ml (9). The determination of hemolytic units results from a hemolysis test with sheep erythrocytes. In short, erythrocytes were exposed to dilution series of purified pneumolysin, and hemolysis was determined. 1 hemolytic unit results in 50% hemolysis (41).

**Plasmids and transient transfection procedures.** HEK-293 cells were cultured in 12-well plates with DMEM supplemented with 10% FCS. Subconfluent cells were cotransfected by the calcium phosphate precipitation method according to the manufacturer’s instructions (Clontech, Palo Alto, CA) with 0.2 μg of NF-κB-dependent luciferase reporter (27), 0.2 μg of respiratory syncytial virus (RSV) β-galactosidase plasmid, 0.2 μg human (h) TLR4 expression vector or 0.05 μg hTLR2, 0.6 μg hTLR1, 0.02 μg CD25 [all generously provided by Carsten Kirstchag, Munich, Germany, and Tularak, San Francisco, CA (25, 46), or 0.2 μg hTLR6 (kindly provided by Dr. Shizou Akira, University of Osaka, Osaka, Japan) and a dominant-negative (dn) mutant of MyD88, or RhoN19, RacN17, and Cdc42 (generous gifts of Drs. Marta Muzio and Anne Ridley, respectively) expression vector]. We kept DNA concentration constant by adding an appropriate amount of control vector. All transfections were performed in duplicate. Cells were incubated with R6x for 6 h on the following day. Luciferase activity was measured by using Luciferase Reporter-Gene Assay (Promega, Mannheim, Germany), and results were normalized for transfection efficiency with values obtained by RSV-β-galactosidase as described previously (32). Morphological changes in HEK-293 cells transfected with dn RhoA/Rac1/Cdc42 were analyzed by phase-contrast microscopy before bacteria stimulation.

**IL-8, IL-1β, TNF-α ELISA.** Confluent BEAS-2B or transfected HEK-293 cells were stimulated as indicated in a humidified atmosphere. After incubation supernatants were collected and processed for cytokine quantification by sandwich ELISA as described previously (20).

**RT-PCR.** For analysis of TLR1, 2, 4, 6, and GAPDH gene expression in BEAS-2B cells, total RNA was isolated with RNasy Mini kit (Qiagen, Hilden, Germany) and reverse transcribed using AMV reverse transcriptase (Promega, Heidelberg, Germany). Generated cDNA was amplified by PCR with specific primers for TLR1 (5'-CGG GAT CCA CAC TGA GAT TGC CCA-3', 5'-CGG ATT CCA CTT GGA GGA TTC TAA-3'), TLR2 (5'-GCC AGA TTG TTT ATT GAT TGG-3', 5'-TGT TGG TTC TCC AGC TGC TG-3'), TLR4 (5'-TAC AGC AGG TGG TGG CTG TG-3', 5'-CCA GAA CCA AAC GAT GGA CT-3'), TLR6 (5'-AGA ACT CAC CAG AGG TCC AAC C-3', 5'-GAA GGC ATG ATA TTC AGC AG-3'), and GAPDH (5'-CCA CCC ATG GCA AAT TCC ATG GCA-3', 5'-TCT AGA CGG CAT GTC AGG TCC-3'). After 32 amplification cycles, PCR products were analyzed on 1.5% agarose gels, stained with ethidium bromide, and subsequently visualized. To confirm use of equal amounts of RNA in each experiment, all samples were checked for GAPDH mRNA expression.

**FACS analysis of CHO cells.** CHO-TLR4/CD14 cells were plated at a density of 2 × 10^5 cells/well in six-well dishes. The following day, cells were stimulated as indicated in Ham’s F-12 medium containing 10% FBS (total volume of 2 ml/well). After 18 h, the cells were harvested with trypsin-EDTA and labeled with allopheco- cyanin anti-CD25 in PBS/1% FBS for 30 min on ice. After labeling, the cells were washed once and resuspended in PBS/1% FBS. The cells were analyzed by flow cytometry using a FACScalibur (Becton Dickinson) (28).

**Chromatin immunoprecipitation.** BEAS-2B cells were stimulated with 10^7 cfu/ml S. pneumoniae R6x for 2.5 h, culture medium was removed, and 1% formaldehyde was added. After 1 min, cells were washed in ice-cold 0.125 M glycine in PBS and then rapidly collected in ice-cold PBS, centrifuged, and washed twice with ice-cold PBS. Cells were lysed by radioimmunoprecipitation assay (RIPA) buffer [10 mM Tris, pH 7.5, 150 mM NaCl, 1% Nonidet P (NP)-40, 1% desoxycholic acid, 0.1% SDS, 1 mM EDTA, and 1% aprotinin], and the chromatin was sheared by sonication. Lysates were cleared by centrifugation, and supernatants were stored in aliquots at −80°C until further use. Antibodies used for immunoprecipitation were purchased from Cell Signaling, Beverly, MA (P-c-Jun and P-Ser276, P-akt (Ser473), and GAPDH (5'-CCA CCC ATG GCA AAT TCC ATG GCA-3'). After 32 amplification cycles, PCR products were analyzed on 1.5% agarose gels, stained with ethidium bromide, and subsequently visualized. The amounts of input DNA was controlled by RT-PCR and gel electrophoresis (42).

The following promoter-specific primers were used: IL-8 sense 5'-AAG AAA ACT TTC GTC ATA CTC CG-3', antisense 5'-TGG CTT TTT ATA TCA TCA CCC TAC-3'.

**Statistical methods.** Data are shown as means ± SE of at least three independent experiments. A one-way ANOVA was used for data of Figs. 1, A–D; 2, B–E; 3, A–C; and 4, A–D. Main effects were then compared by a Newman-Keuls posttest. P < 0.01 was considered to be significant and indicated by asterisks.
RESULTS

*S. pneumoniae* induced Rac1-dependent expression of IL-8 in human bronchial epithelial cells. Recognition of pneumococci by tracheobronchial epithelium and subsequent expression of proinflammatory cytokines may contribute significantly to the prominent leukocyte recruitment observed in pneumococci pneumonia. Therefore, we assessed the potential of pneumococci-induced IL-8 expression in human bronchial epithelial cells. Bronchial epithelial BEAS-2B cells stimulated with *S. pneumoniae* R6x displayed dose-dependently enhanced IL-8 expression (Fig. 1A). In terms of BEAS-2B cell stimulation, *S. pneumoniae* turned out to be as effective as 100 ng/ml TNF-α, which resulted in an IL-8 release of ~20 ng/ml in the cell supernatant (Fig. 1C). To rule out the possibility that pneumococci-related IL-8 liberation was due to secondary autocrine cytokine stimulation via epithelially generated TNF-α or IL-1β, TNF-α and IL-1β release was also analyzed in *S. pneumoniae*-infected BEAS-2B cells. Although TNF-α increased in parallel to IL-8, significant IL-1β release was detected only after 10 h of cell infection (data not shown), suggesting that secondary effects of TNF-α and IL-1β are not important in the experimental system used. To test the hypothesis that Rho-GTPases might be involved, we used the specific Rac1 inhibitor Nsc. The compound was shown to specifically block activation of Rac1, but not RhoA or Cdc42, at concentrations between 50 and 200 μM (13). Nsc dose-dependently reduced IL-8 release in pneumococci-stimulated BEAS-2B cells (Fig. 1B). Nsc also reduced TNF-α- or IL-1β-related IL-8 generation (Fig. 1, C and D, respectively). Thus Rac1 seems to be critically involved in pneumococci-related IL-8 release by cultured human bronchial epithelial BEAS-2B cells.

*S. pneumoniae* induced NF-κB-dependent gene transcription by TLR2, and pneumolysin by TLR4. To analyze epithelial recognition of *S. pneumoniae* in more detail, we studied TLR mRNA expression. Unstimulated BEAS-2B cells expressed TLR4 and TLR6, whereas TLR1 and TLR2 mRNA was barely detectable (Fig. 2A). Infection of BEAS-2B cells with pneumococci time dependently induced expression of TLR2 mRNA after 6 h and TLR1 mRNA after 12 h. In contrast, TLR4 and TLR6 mRNA expression was constitutively high in control and infected cells.

Next we analyzed the role of TLR receptors for pneumococci recognition by transfecting HEK-293 cells with TLR2 or TLR4. Expression of a NF-κB-dependent reporter gene in *S. pneumoniae* (10^6 cfu/ml)-, pneumolysin (0.1 and 1 μg/ml)-, or positive control (peptidoglycan or LPS, respectively)-exposed cells was measured. Neither pneumococci nor pneumolysin activated NF-κB-dependent gene transcription in HEK-293 cells without transfected TLR2 or TLR4 (Fig. 2C), but reporter gene expression was strongly activated by 10 ng/ml TNF-α (data not shown). *S. pneumoniae*-induced NF-κB activity occurred exclusively in TLR2-expressing cells (Fig. 2B) and pneumolysin-induced NF-κB activity occurred exclusively in TLR4-expressing HEK-293 cells (Fig. 2D).

We verified recognition of pneumolysin by TLR4 using CD14- and hTLR4-positive CHO cells expressing an NF-κB-dependent reporter gene. These cells are unresponsive to TLR2 activation due to a single base-pair deletion in the *tlr2* gene (25). NF-κB-dependent reporter gene expression was noted in CHO-TLR4/CD14 cells upon stimulation with LPS (100 ng/ml) or pneumolysin (1 μg/ml) (Fig. 2F).

To exclude effects of possibly contaminating LPS in the pneumolysin preparation we made use of polymyxin B (PMB). Preincubation of LPS (100 ng/ml) with 10 μg/ml PMB blocked activation of NF-κB in hTLR4-transfected HEK-293 cells almost completely. In contrast, PMB had no inhibitory effect on pneumolysin-induced activation of NF-κB in these cells (Fig. 2E). Thus relevant LPS contamination seems to be unlikely in the pneumolysin preparation used.

TLR1 and TLR2 synergistically induced *S. pneumoniae*-dependent NF-κB-dependent gene transcription, but TLR4, CD14, or LBP did not enhance recognition of *S. pneumoniae*. Binding of bacteria to TLR homo- or heterodimers is considered to activate host cells (1). We noted expression of a reporter gene activated by the important proinflammatory transcription factor NF-κB in pneumococci-stimulated HEK-293 cells overexpressing TLR2, but not TLR1 or TLR6 alone (Fig. 3A). TLR2 is reported to recognize pathogens in cooperation with other TLR receptors. TLR2-dependent NF-κB activation by pneumococci was synergistically enhanced by coexpression of TLR1. TLR6 did not boost pneumococci-induced reporter gene levels in context with TLR2. Thus TLR1 and TLR2 seem to be synergistically involved in pneumococci-induced cell activation.

Pneumococci did not induce NF-κB-dependent gene transcription in HEK-293 by TLR4 alone (Fig. 2D). We performed cotransfection experiments with TLR2 and TLR4 to see whether they act synergistically with respect to pneumococci-dependent cell activation. However, HEK-293 cells cotrans-
Infected with TLR2 and TLR4 displayed no significant change in pneumococci-induced NF-κB-activation above effects seen in TLR2-carrying cells (Fig. 3B).

The recognition of LPS by TLR4 and some other bacterial surface molecules, e.g., LTA by TLR2, is assisted by LBP and CD14 (43). To test whether this is also the case for living pneumococci and human TLR2, we stimulated TLR2-expressing HEK-293 cells with *S. pneumoniae* in the presence of FCS or recombinant LBP and transfection of CD14, respectively, and measured NF-κB-dependent reporter gene expression (Fig. 3C). Although there was a trend for enhanced NF-κB activity when FCS or a combination of CD14 and LBP were added, no significant effect of serum, CD14, or LBP on the recognition of viable pneumococci by TLR2 was found within the dose and time frame tested.

*S. pneumoniae* induced MyD88-, PI3K- and Rac1-dependent gene transcription and recruitment of phosphorylated p65 to the IL-8 gene promoter. Next, we analyzed pneumococci-induced signal transduction. MyD88, PI3K, and Rho-GTPases were considered as part of the TLR2 pathway (22). Therefore, hTLR2-expressing HEK-293 cells were transfected with dn mutants of MyD88, RhoA, Rac1, and Cdc42, stimulated with *S. pneumoniae*, and NF-κB-dependent reporter gene expression was measured. dn-MyD88 (Fig. 4A), dn-Rac, and dn-Cdc42 (Fig. 4B) reduced pneumococci-induced NF-κB activity, whereas dn-RhoA had no inhibitory effect, although it induced specific morphological changes in transfected HEK-293 cells (data not shown). In addition, we measured IL-8 release by transfected HEK-293 cells and similarly found that Rac1 and Cdc42 but not RhoA were necessary for induction of IL-8 release by *S. pneumoniae*-infected cells (Fig. 4C).

As PI3K is considered as an upstream activator of Rho proteins, we verified the involvement of this pathway in pneumococci-infected human bronchial epithelial cells: Blocking of PI3K in BEAS-2B cells by specific inhibitor LY-294002 dose dependently reduced IL-8 release (Fig. 4D).

To understand Rac1 involvement in IL-8 expression on a more molecular level, we measured transcription factor recruitment to the native IL-8 gene promoter by chromatin immunoprecipitation (Fig. 4E). *S. pneumoniae* infection of BEAS-2B cells induced binding of activator protein (AP)-1 subunit c-Jun phosphorylated on Thr^32/39, whereas TLR2 activation induced c-Jun phosphorylation on Ser^63. The inactive AP-1 subunit p50 bound to the IL-8 promoter only in TLR2-carrying cells.

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**Fig. 2.** *S. pneumoniae* (S.p.) and pneumolysin (Ply) activated NF-κB-dependent gene transcription via Toll-like receptor (TLR) 2 and TLR4, respectively. A: BEAS-2B cells were infected with 10⁶ cfu/ml R6x as indicated, and mRNA for TLR1, TLR2, TLR4, TLR6, and GAPDH was detected by RT-PCR. One representative gel out of 3 is shown. HEK-293 cells were transfected with plasmids encoding an NF-κB-dependent luciferase reporter, RSV β-galactosidase, and as indicated with TLR2 (B), TLR4 (D), or not transfected with a TLR receptor (C). Cells were exposed to R6x S.p. (10⁶ cfu/ml) or Ply (0.1–1 μg/ml) for 6 h, and luciferase activity was determined and normalized on β-galactosidase activity. *P < 0.01 compared with control. E: HEK-293 cells were transfected with plasmids encoding an NF-κB-dependent luciferase reporter gene, RSV β-galactosidase, and TLR4. Ply (1 μg/ml) or LPS (100 ng/ml) was preincubated with polymyxin B (PMB, 10 μg/ml for 1 h) and then given to TLR4-transfected HEK-293 cells for 6 h. Luciferase activity was determined and normalized on β-galactosidase activity. F: CHO-TLR4/CD14 cells were stimulated with Ply (1 μg/ml) or LPS (100 ng/ml) for 18 h, and CD25 reporter gene expression was measured by FACS analysis. C, control; PGN, peptidoglycan; RLU, relative light units. Graphs from 1 representative experiment out of 3 are shown. Numbers represent fluorescence median of 3 experiments (fluorescence means ± SE).
serine 63, and NF-κB subunit p65/RelA phosphorylated on serine 276 (Fig. 4F). Recruitment of serine 276-phosphorylated p65/RelA was blocked, and p65/RelA binding was reduced by inhibition of Rac1, whereas binding of phosphorylated c-Jun remained unaffected by Nsc in pneumococci-infected BEAS-2B cells.

**DISCUSSION**

In this study we found activation of pneumococci-infected lung epithelial cells by TLR1/2-induced PI3K and Rac1 signal transduction. *S. pneumoniae* stimulated Rac1-dependent IL-8-release by human bronchial epithelial cells. Pneumococci enhanced TLR1 and TLR2 expression in these cells. Viable pneumococci were synergistically recognized by TLR1 and TLR2, whereas pneumolysin was only recognized by TLR4. TLR4, LBP, or CD14 displayed no significant effect on pneumococci-induced TLR2-dependent cell activation. Pneumococci-induced signaling involved MyD88, PI3K, Rac1, and Cdc42. Recruitment of NF-κB subunit p65/RelA to the IL-8 promoter was regulated by Rac1-dependent phosphorylation of serine 276, whereas Rac1 did not influence pneumococci-induced binding of phosphorylated c-Jun.

Tracheobronchial epithelium is part of the important mechanical barrier of the lung against invading pathogens and represents the first line of defense of the innate immune system in the lung. We analyzed pneumococci-induced activation of the well-established human bronchial epithelial cell line BEAS-2B, addressing the chemotactic IL-8 as a model cytokine, which was suggested to play an important role in lung inflammation (48).

Rho-GTPases have been shown to signal downstream of TLRs, e.g., TLR2 (3) in HEK-293 and THP-1 cells and TLR4 (20) in human endothelial cells. These pathways seem to include MyD88 and PI3K and *S. pneumoniae*-induced gene transcription in pneumococci-exposed epithelium. Transfection of dn Rac1 and Cdc42 as well as the specific Rac1 inhibitor Nsc (13) blocked NF-κB activity and IL-8 release, whereas RhoA was not involved. Interestingly, Rac1-dependent IL-8 release was also observed in intestinal epithelial cells infected with gram-negative *Yersinia enterocolitica* (15). Moreover, contribution of Rac1 in NF-κB-activation has been demonstrated after stimulation with heat-killed *Staphylococcus aureus* (3). In these experiments Cdc42 was also activated but without effect on NF-κB activation (3). Because Rho proteins, in particular Rac1, also participated in IL-1β (45)- and TNF-α (19)-dependent NF-κB activation in various cells (also in BEAS-2B, as shown here) these GTPases must be considered as important signaling mediators in inflammatory reactions. However, dn mutants as well as chemical inhibitors have their technical limitations, and further studies employing Rac1-defective cells are needed.

Different mechanisms have been suggested for Rho-GTPase-dependent activation of NF-κB: Arbibe et al. (3) implicated TLR2-/PI3K-/Rac1-dependent phosphorylation of NF-κB, p65/RelA subunit of NF-κB was indeed phosphorylated PI3K dependently at serine 536 after TLR2 stimulation (47), but involvement of Rac1 was not addressed in that study. We show herein that Rac1 inhibition reduced pneumococci-induced binding of p65 to the IL-8 promoter and blocked phosphorylation of p65 on serine 276. This phosphorylation site has been implicated in DNA binding of NF-κB (8). In contrast, the AP-1 subunit c-Jun, which was phosphorylated on serine 63 in pneumococci-stimulated epithelial cells, was recruited to the IL-8 promoter independently of Rac1, indicating an NF-κB-specific mechanism. In other studies, RhoA was shown to be involved in TLR2-dependent phosphorylation of p65/RelA on serine 311 by PKCζ (52) and thrombin-induced phosphorylation of p65/RelA on serine 536 by IKKβ (2) in different cells and stimulation conditions.
In unstimulated epithelial cells, strong mRNA expression of TLR4 and TLR6 was noted, whereas TLR1 and TLR2 expression seemed to be low. Experiments with pneumococci-stimulated BEAS-2B cells demonstrated increased TLR1 and 2 expression and unchanged levels of TLR4 and TLR6 mRNA. Armstrong et al. (4) recently reported modulation of TLR2 and TLR4 expression by LTA and LPS in human type II alveolar epithelial cells.

TLR2 seemed to be involved in sensing of pneumococci (43), and mice lacking TLR2 were highly susceptible to pneumococcal meningitis due to lower bacterial clearance and enhanced inflammation (10). However, although TLR2 contributed significantly to the early inflammatory response in murine pneumococcal pneumonia, it did not contribute to antibacterial defense per se (26). Recent reports have given evidence that TLR2 synergizes with TLR1 (57) or TLR6 (50) to activate cells. In our model, pneumococci-induced NF-κB activation was much stronger in cells expressing TLR1 and TLR2 compared with cells transfected with TLR2 alone or together with TLR6. TLR1 and TLR2 were found to interact physically (51) and form heterodimers for signal transduction (38). Thus subsequent upregulation of these pattern recognition receptors in pneumococci-infected epithelial cells may sensitize the epithelial barrier to pneumococci infection, thereby strengthening innate immune response. Pneumolysin activated TLR4-expressing cells, an observation in accordance with Malley et al. (29), who demonstrated an impact of TLR4 on pneumococcal clearance in mouse infection. Interestingly, viable pneumococci were not recognized by TLR4 in our model, nor did TLR4 synergize with TLR2 in S. pneumoniae recognition, most likely because pneumolysin is localized in the bacterial cytosol and only released by autolysis action (21). In addition to pathogen recognition receptors localized at the surface, intracellular detection of invading pneumococci by Nod2/CARD15 proteins may participate in target cell activation as shown recently (32).

LBP and CD14 are important cofactors for LPS sensing by the immune system and also facilitate recognition of pneumococcal LTA (43, 55). Moreover, LBP has become a very actively investigated molecule in sepsis, as well as other infectious diseases and is considered as therapeutic target (59). Weber et al. (55) found, in a mouse model of pneumococcal meningitis, involvement of LBP in pathogenesis. Therefore, we analyzed the impact of LBP and CD14 on recognition of viable pneumococci by TLR2-expressing cells. However, in our model, we did not find significantly increased TLR2-dependent NF-κB activation by pneumococci in the presence of CD14 or LBP. LBP knockout mice accordingly displayed increased susceptibility to gram-negative Haemophilus influenzae infection, but similarly sufficient immune reaction against S. pneumoniae (6) or TLR2 stimulus nonmannose-capped lipoarabinomannan (56). Therefore, the function of LBP in cell activation seems to be stimulus and cell dependent.

In summary, S. pneumoniae activated human bronchial epithelial cells in vitro by TLR1/2 and a PI3K-Rac1-NF-κB-dependent pathway, whereas pneumolysin stimulated TLR4 signaling. Further studies on Rho protein involvement in pneumococcal pneumonia are required and may pave the way to the development of new therapeutic strategies.

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