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MyD88 and TNF Receptor-Associated Factor 6 Are Critical Signal Transducers in Helicobacter pylori-Infected Human Epithelial Cells

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Helicobacter pylori induces NF-κB activation, leading to mucosal inflammation via cag pathogenicity island. Although recent studies have implicated several candidate proteins of both H. pylori and host, the molecular mechanism by which H. pylori activates NF-κB remains unclear. The aim of this study was to analyze the mechanism of cag pathogenicity island-mediated NF-κB activation in epithelial cells. The responses of human cell lines and mouse embryonic fibroblasts to infection with wild-type H. pylori or cagE mutant were investigated. The effect of small interfering RNAs (siRNAs) for several NF-κB signaling intermediate molecules was evaluated in H. pylori-induced IκBα phosphorylation and IL-8 production. Protein interactions of exogenously expressed TNFR-associated factor 6 (TRAF6) and MyD88 or receptor-interacting protein 2 and nucleotide-binding oligomerization domain 1 or those of endogenous IκB kinase, TGF-β-activated kinase 1 (TAK1), and TRAF6 were assessed by immunoprecipitation. Cag pathogenicity island-dependent NF-κB activation was observed in human cell lines, but not in mouse fibroblasts. In human epithelial cells, H. pylori-induced IκBα phosphorylation and IL-8 production were severely inhibited by siRNAs directed against TAK1, TRAF6, and MyD88. In contrast, siRNAs for TRAF2, IL-1R-associated kinases 1 and 4, and cell surface receptor proteins did not affect these responses. H. pylori infection greatly enhanced MyD88 and TRAF6 complex formation in a cag-dependent manner, but did not enhance Nod1 and receptor-interacting protein 2 complex formation. H. pylori also induced TAK1 and TRAF6 complexes. These results suggest that the cag pathogenicity island of H. pylori is a cell type-specific NF-κB activator. TAK1, TRAF6, and MyD88 are important signal transducers in H. pylori-infected human epithelial cells. The Journal of Immunology, 2006, 176: 3796–3803.
strains activate NF-κB. In addition, NF-κB hyperactivity has been reported for epithelial cell carcinoma, such as breast cancer (23). Thus, detailed investigation of this signaling pathway will increase our understanding of the pathogenesis of malignancies and should be useful in mapping out therapeutic strategies.

Although many studies have reported that cag-positive H. pylori strains activate NF-κB, the mechanisms by which these bacteria activate the cellular signaling cascades remain unclear. Recently, peptidoglycan injected by H. pylori infection has been shown to interact with the cellular membrane nucleotide-binding oligomerization domain 1 (Nod1), leading to activation of the NF-κB signaling pathway (24). However, it remains unsolved whether Nod1 is the main and only signaling pathway for NF-κB activation by H. pylori. To obtain a better understanding of H. pylori pathogenesis, we have examined the molecular pathway of NF-κB activation induced by cag-positive H. pylori.

Materials and Methods

Cell lines and H. pylori strains

The AGS human gastric cancer cell line was maintained at 37°C in 5% CO₂ in Ham’s F-12 medium that was supplemented with 10% FBS as described previously (25). HeLa cells and HEK293T cells were purchased from the Riken Cell Bank and maintained as described previously (26, 27). Mouse embryonic fibroblasts (MEFs) were obtained from 13.5-day-old embryos of C57BL/6 mice. The cells were cultured at 37°C in 5% CO₂ in DMEM that contained 10% FBS supplemented with penicillin, streptomycin, glutamine, sodium pyruvate, and nonessential amino acids.

The type 1 H. pylori strains TN2 and 26695 were maintained under microaerophilic conditions in Brucella broth that was supplemented with 5% horse serum. TN2-(Δage)4, an isogenic mutant that lacks a functional type IV secretion system, was grown on kanamycin-containing plates (25 μg/ml) and maintained in Brucella broth without antibiotics (16, 26). These bacterial strains were washed with PBS, and the concentrations were estimated using OD₅₀₀ = 0.1 as equivalent to 4 × 10⁶ CFU H. pylori. The bacteria were resuspended in Ham’s F-12 or DMEM before infection.

Cultured cells were washed twice with PBS and supplemented with fresh medium without antibiotics, followed by infection with H. pylori for the indicated time periods at a multiplicity of infection of 100.

Small-interfering RNA (siRNA), plasmids, and transfection

RNA oligonucleotides for siRNA were synthesized (Qiagen) with the following sequences for nonsilencing control, 5'-UUCUCCGAACGUGUCACGU-3'; for TGF-β-activated kinase 1 (TAK1), 5'-UUGGCUAU CUACACAGUGA-3' (28); for TNFR-associated factor 6 (TRAPF6), 5'-AAGGAGCAACUCUGUGAAGU-3'; for TRAP2, 5'-AAGGAGCACUUGCUCAAGGA-3'; for MyD88, 5'-AAGGACCAUGAGAAGAGAAGUC-3'; and for IL-1R-associated kinase 1 (IRAK1), 5'-AAGGUUCCAUCCUCAGCCUC-3' (29). The SiGENOME mixture for Nod1, TLR2, TLR4, TLR9, and IL-1R accessory protein (AcP) was obtained from Dharmacon. Silencer-validated siRNA for receptor-interacting protein (RIP2; ID no. 456) was purchased from Ambion. All siRNA transfections were performed with LipofectAMINE 2000 reagent (Invitrogen Life Technologies). All siRNAs were tested and verified as reducing expression by > 80% protein reduction in AGS cells by immunoblot analysis or reducing expression of > 50% of mRNA by real-time PCR when appropriate. Ab was not available. For the AGS cells, 100 nM of each siRNA was transfected 48 h before H. pylori infection.

The pNF-κB-luciferase (pNF-κB-Luc), pRL-thymidine kinase (pRL-TK), and Flag-TRAP6 expression vectors were used as described previously (16). The pTREx2-hemagglutinin (HA)-MyD88 and pcDNA3.1 Myc-RIP2 were generated by RT-PCR with cDNA obtained from AGS cells. HA-Nod1 was cloned by PCR from the cDNA for Nod1, which was previously (16). The pTriEx-2 hemagglutinin (HA)-MyD88 and pcDNA3.1 TK), and Flag-TRAF6 expression vectors were used as described previously (25). HeLa cells and HEK293T cells were purchased from Sigma-Aldrich. The pSilencer vector, which is a cytomegalovirus (CMV)-based vector, was used as the control construct. For TGF-β, the AGS human gastric cancer cell line was maintained in 5% CO₂ in DMEM with 10% FBS (EuroClone) supplemented with 1 mM sodium pyruvate, 10 μg/ml leupeptin, and 1 mM PMSF. The cell lysates were centrifuged at 10,000 × g for 10 min at 4°C. Equal amounts of the cell lysates were separated by SDS-PAGE and transferred to a polyvinylidene difluoride membrane. The membrane was probed with the indicated primary Ab, followed by HRP-conjugated secondary Ab, and was developed using the ECL Plus Kit (Amersham Biosciences). The membranes were stripped and rebotted to confirm equal loading of the samples. Each immunoblot experiment was performed more than twice, and a representative image is shown.

Immunoprecipitation

AGS cells were cultured in 6-cm diameter dishes and stimulated with H. pylori, IL-1β, or TNF-α for the indicated periods of time. Where indicated, Flag-TRAP6 (0.5 μg) and HA-MyD88 (1 μg) or Myc-RIP2 (0.5 μg) and HA-Nod1 (1 μg) were transfected into the cells 24 h before stimulation. Aliquots (300 μl) of the cell lysates were collected as described above. The cell lysates were immunoprecipitated at 4°C with 5 μl of agrose-conjugated Flag or Myc Ab overnight or with polyclonal TAK1 Ab (2 μl) overnight, followed by a 1-h incubation with protein G-Sepharose beads (Amersham Biosciences). The precipitates were washed three times with ice-cold Triton X-100 buffer, boiled in SDS sample buffer for 5 min, and immunoblotted with the indicated Abs.

Quantification of chemokines by ELISA

The IL-8 levels in the culture supernatants were measured by ELISA as described by the manufacturer (Technne). AGS or HeLa cells plated in 24-well plates were transfected with siRNAs for 48 h and infected with H. pylori for an additional 12 h. The culture supernatants were aspirated and stored at −70°C until assayed by ELISA. The concentration of IL-8 was determined using a standard curve obtained with rIL-8 protein. The values were determined in more than three independent experiments and are represented as the mean ± SD.

Statistical methods

Statistical analysis was performed using Student’s t test and two-sided or one-way ANOVA with Dunnett’s multiple comparison. Differences were considered statistically significant at p < 0.05.

Results

The cag PAI system is required for NF-κB activation in cells that do not respond to LPS stimulation

Initially, we investigated the specificity of cag PAl for NF-κB activation using various types of cultured cells. Consistent with previous reports (12, 32), 12-h stimulation with the cag-positive H. pylori strains activated NF-κB in AGS cells, which are representative of gastric cancer cell lines (Fig. 1A, bottom panel). In accordance with the levels of NF-κB activity measured in the reporter assay, wild-type H. pylori, TNF-α, and IL-1β increased the
levels of p100 protein, which is a known target gene of NF-κB, in 12 h (Fig. 1A, top panel). This cell line was unresponsive to stimulation with either LPS or the H. pylori cag mutant strain in terms of both NF-κB activation and production of p100 protein. We also tested the human epithelial cell lines HeLa and HEK293T and found that wild-type H. pylori, but neither cag mutant H. pylori nor LPS, activated NF-κB reporter and p100 protein production in these cells (data not shown). In contrast to these human epithelial cells, MEFs were highly responsive to both the wild-type and mutant H. pylori strains in terms of NF-κB activation and target protein production (Fig. 1B). MEFs were also sensitive to LPS.

We evaluated in these cells the level of IκBα phosphorylation, which is a key signaling event for classical NF-κB activation. Once again, the wild-type H. pylori, but neither the cagE mutant nor LPS, induced IκBα phosphorylation in AGS (Fig. 1C) and HEK293 (data not shown) cells. However, in MEFs, all these stimuli strongly induced IκBα phosphorylation in a time-dependent manner (Fig. 1D). When we used lymphocytes from C57BL/6 mice or the human lymphocyte cell line IM-9, we observed similar results, i.e., both H. pylori mutant and LPS induced IκBα phosphorylation to the same extent as wild-type H. pylori (data not shown). These results suggest that the cag PAI system of H. pylori is a specialized bacterial component for inducing NF-κB activity in epithelial cells that are essentially unresponsive to LPS.

**FIGURE 2.** TAK1 and TRAF6 are required for H. pylori-induced NF-κB activation in human epithelial cells. AGS cells were treated with siRNA for TAK1 (A), TRAF6 (B), and TRAF2 (C), followed by stimulation with IL-1β (A and B), TNF-α (C), and H. pylori (TN2) for the indicated periods of time. The cell lysates were subjected to immunoblot analysis for phospho-IκBα, phospho-JNK, or other proteins.

**TAK1 and TRAF6 are required for cag PAI-mediated NF-κB activation**

To address the mechanism of cag PAI-mediated NF-κB activation, we used siRNAs to block the signaling intermediates in this pathway. Because TAK1 is known to transduce signals from the IL-1R or TNFR to IKK (28, 33), we initially transfected siRNA for TAK1 into AGS cells and assessed the effect on NF-κB activation. As shown in Fig. 2A, TAK1 siRNA effectively blocked NF-κB activation, TAK1 and TRAF6 are required for cag PAI-mediated NF-κB activation.

Once again, the wild-type H. pylori, but neither the cagE mutant nor LPS, induced IκBα phosphorylation in AGS (Fig. 1C) and HEK293 (data not shown) cells. However, in MEFs, all these stimuli strongly induced IκBα phosphorylation in a time-dependent manner (Fig. 1D). When we used lymphocytes from C57BL/6 mice or the human lymphocyte cell line IM-9, we observed similar results, i.e., both H. pylori mutant and LPS induced IκBα phosphorylation to the same extent as wild-type H. pylori (data not shown). These results suggest that the cag PAI system of H. pylori...
for TRAF6 inhibited the phosphorylation of both IkBα and JNK induced by *H. pylori* in AGS cells. The siRNA for TRAF6 had negligible effects on IkBα phosphorylation induced by TNF-α (data not shown). In contrast, TRAF2 silencing did not affect *H. pylori* infection-mediated signaling activation, although TNF-α-induced signaling was effectively down-regulated (Fig. 2C). The specificities of TRAF6-dependent and TRAF2-independent NF-κB and JNK signaling activation by *H. pylori* were also confirmed in HeLa cells (data not shown).

**MyD88 is required for cag PAI-mediated NF-κB activation**

TRAF6 is an important signal transducer in IL-1R signaling and TLR signaling, both of which pathways involve MyD88 as an adaptor molecule (reviewed in Refs. 35 and 36). Therefore, we assessed the involvement of this adaptor molecule in *H. pylori*-infected AGS cells. As shown in Fig. 3A, MyD88 silencing effectively inhibited the phosphorylation of IkBα and JNK caused by either *H. pylori* infection or IL-1 stimulation. In contrast, silencing of TRADD, the adaptor molecule for TNFR signaling, did not affect *H. pylori*-mediated signaling activation (data not shown). We also confirmed that siRNA for MyD88 did not reduce TNF-mediated IkBα phosphorylation (data not shown). These results indicate that cag PAI-dependent NF-κB activation by *H. pylori* requires the MyD88 protein.

We also established two stable AGS cell lines; AGS-B4 showed increased MyD88 protein due to exogenous CAG promoter-driven expression, whereas AGS-71 showed decreased MyD88 protein expression due to U6 promoter-driven MyD88 silencing. *H. pylori* induced a higher level of IkBα phosphorylation in AGS-B4 cells than in control (AGS-C1) cells (Fig. 3B). In contrast, *H. pylori* induced a lower level of phosphorylation in AGS-71 cells. This result represents additional evidence for the involvement of the MyD88 adaptor protein in cag PAI-mediated NF-κB activation in AGS cells.

**Analysis of the signaling complex in *H. pylori*-infected epithelial cells**

We next investigated the involvement of TAK1, TRAF6, and MyD88 in the *H. pylori*-mediated signalosome by assessing the intermolecular interactions. Initially, we transfected tagged MyD88 and TRAF6 into AGS cells and performed immunoprecipitation. As with IL-1 stimulation, *H. pylori* induced complex formation between HA-tagged MyD88 and Flag-tagged TRAF6 within 30 min of infection (Fig. 4A). Importantly, this interaction was induced only by the wild-type *H. pylori* and not by the cagE mutant (Fig. 4B). This suggests that these molecules are involved in cag PAI-mediated cell signaling.

We also investigated the role of TAK1 in the signalosome. As shown in Fig. 4C, endogenous TRAF6 formed a complex with TAK1 upon *H. pylori* infection. Furthermore, TAK1 immunoprecipitated with IKKγ, which is a critical regulator of the IKK complex, in a *H. pylori* infection-dependent manner (data not shown). These results indicate that TAK1, TRAF6, and MyD88 are important molecules that link cag-positive *H. pylori* infection to the IKK complex in AGS cells.

**FIGURE 3.** MyD88 is required for *H. pylori*-induced NF-κB activation in human epithelial cells. A, AGS cells were treated with siRNA for MyD88 and subsequently stimulated with IL-1β and *H. pylori* (TN2) for the indicated periods of time. The cell lysates were analyzed by immunoblotting, as described in Fig. 2B. Stable cell lines for control (AGS-C1), MyD88 overexpression (AGS-B4), and MyD88 knockdown (AGS-71) were infected with *H. pylori* (TN2), and IkBα phosphorylation was assayed (top panel). The MyD88 protein level is also shown (middle panel). The arrow and arrowhead indicate endogenous and HA-tagged MyD88 proteins, respectively.

**FIGURE 4.** Analysis of the molecular interactions induced by *H. pylori*. A, AGS cells were cultured in a 6-cm diameter dish and transfected with 1 μg of HA-MyD88 and 0.5 μg of Flag-TRAF6 vector for 24 h. The cells were then treated with TNF-α, IL-1β, and *H. pylori* (TN2) for the indicated periods of time. The cell lysates were immunoprecipitated with anti-Flag Ab. The immunoprecipitated proteins (left panel) and total cell lysates (right panel) were analyzed by immunoblotting with the indicated Ab. B, AGS cells, which were transfected with HA-MyD88 and Flag-TRAF6, were infected with *H. pylori* (TN2 or TN2-ΔcagE). Immunoprecipitation and immunoblotting were performed as described in A. C, AGS cells were cultured in a 6-cm diameter dish and infected with *H. pylori* for the indicated time periods. The cell lysates were immunoprecipitated with 2 μl of polyclonal anti-TAK1 Ab. The immunoprecipitated proteins and total cell lysates were immunoblotted with the anti-TRAF6 Ab.
TAK1, TRAF6, and MyD88 are important for IL-8 secretion from H. pylori-infected epithelial cells

Because the above siRNAs experiments involved only short postinfection incubation periods of 30–90 min, we could not exclude the possibility that these molecules are independent of H. pylori-induced cell signaling at later time points. Therefore, we investigated the effects of these siRNAs on IL-8 chemokine induction, which usually requires several hours. These siRNAs did not reduce IL-8 secretion from noninfected cells (70–200 pg/ml in AGS cells; data not shown). As shown in Fig. 5, coculture with H. pylori for 12 h induced ~2500 pg/ml IL-8 in the control oligonucleotide-transfected cells, whereas it induced only ~800–1400 pg/ml IL-8 in the TAK1-, TRAF6-, or MyD88 siRNA-treated cells (p < 0.01). TRAF2 silencing slightly reduced IL-8 secretion from H. pylori-infected AGS cells; however it was not statistically significant. We found similar IL-8 suppression by siRNA for TAK1, TRAF6, and MyD88 in H. pylori-infected HeLa cells (data not shown). These results indicate that TAK1, TRAF6, and MyD88 are necessary not only for transient phosphorylation of IkBα and JNK, but also for subsequent IL-8 production in AGS cells.

Analysis of molecules that affect the MyD88-TRAF6 complex

MyD88 was established as a signal transducer of IL-1β and TLR signaling pathways. IL-1R, TLRs, IRAK1, and IRAK4 are known to interact with MyD88 and transduce the signal (35–37). Therefore, we examined the involvement of these molecules in H. pylori-induced cell signaling. When we transfected siRNA for IRAK1 or IRAK4 into AGS cells, H. pylori induced levels of IkBα and JNK activation similar to those in control transfected cells (Fig. 6A), which indicates that these kinases are not necessary for H. pylori-induced NF-κB activation. We also investigated cell surface receptors, such as IL-1R and TLRs. However, siRNAs for IL-1R Acp, TLR4, TLR2, and TLR9 did not affect IkBα phosphorylation after H. pylori infection (data not shown). In addition, IL-8 secretion from these cells after 12 h of H. pylori infection was not reduced (Fig. 6B). These results indicate that H. pylori activates NF-κB via MyD88, albeit independently of IL-1R and TLR.

Assessment of the involvement of Nod1

During the course of our study, Viala et al. (24) described Nod1 as a critical regulator of H. pylori cag PAI-mediated signaling. Therefore, we evaluated the contribution of Nod1 to H. pylori-induced cell signaling. We used siRNAs for Nod1 and RIP2, which is an important kinase that links the Nod protein to the IKK complex (30, 38). When we infected cells with H. pylori TN2, we did not observe a decrease in IkBα phosphorylation compared with the control oligonucleotide-transfected cells (Fig. 7A). The levels of Nod1 transcripts were decreased in RT-PCR (data not shown). We also assessed the Nod1-RIP2 interaction by immunoprecipitation analysis. In contrast to the MyD88 and TRAF6 transfection experiment, the HA-Nod1 protein bound to Myc-RIP2 in untreated cells. However, this interaction was not enhanced by H. pylori infection (Fig. 7B). We also measured the levels of IL-8 secretion from these cells (Fig. 7C). H. pylori infection induced ~2500 pg/ml IL-8 in control-transfected AGS cells. Similar levels of IL-8 were observed in Nod1 siRNA-treated cells. H. pylori induced higher levels of IL-8 (3700 pg/ml) in RIP2 siRNA-transfected cells (p < 0.05).

Because Nod1-dependent signaling was shown using the H. pylori 26695 strain (24), we used the 26695 strain as another cag PAI-positive H. pylori strain, to exclude strain specificity. We found that strain 26695-induced IkBα phosphorylation was severely inhibited by the siRNA for MyD88, but not by the siRNA for Nod1 (Fig. 7D). Thus, IkBα phosphorylation induced by H. pylori seems to be independent of Nod1 expression. Collectively, these results suggest that the Nod1-RIP2 pathway is not the principal signaling transducer in H. pylori-infected epithelial cells, at least under our experimental conditions.

Discussion

Since the discovery of the CagA protein and the cag PAI genes, they have become recognized as important markers of H. pylori

![FIGURE 5. TAK1, TRAF6, and MyD88 are required for IL-8 secretion by H. pylori-infected epithelial cells. AGS cells were treated with the indicated siRNAs for 48 h and subsequently infected with H. pylori (TN2) for 12 h. The culture supernatants were examined for IL-8 content by ELISA. The values shown are the mean ± SD from three independent experiments. *p < 0.01, by Student’s t test.](image)

![FIGURE 6. Analysis of MyD88-related proteins in H. pylori-infected epithelial cell signaling. A, AGS cells were transfected with control, IRAK1, or IRAK4 siRNA for 48 h, then infected with H. pylori for the indicated time periods. Immunoblotting was performed with the indicated Abs. B, AGS cells were transfected with the indicated siRNAs, then infected with H. pylori for 12 h. The culture supernatants from three independent experiments were analyzed by ELISA as described in Fig. 5.](image)
virulence (5, 7, 8). Furthermore, strains that carry cag PAI have been revealed to activate various cellular signaling pathways. However, the mechanism by which cag PAI induces intracellular signaling activation has not been fully elucidated. In this report we describe the critical roles of MyD88 and TRAF6 in cag PAI-mediated signal transduction in epithelial cells.

Recently several receptors, such as, TLRs and Nod proteins, have been shown to play a central role in sensing bacterial components in the immune system (reviewed in Refs. 35, 36, 39, and 40). However the functionality and the importance of these bacterial receptors depend on cell types, and those in epithelial cells are as yet relatively undefined (41, 42). Therefore, we used several cell lines to assess cag PAI-mediated signaling transduction and found that cag PAI was especially significant in human epithelial cells. Importantly, LPS treatment had negligible effects on NF-κB activation in these cells. In contrast, in MEFs, the cag mutant H. pylori, as well as LPS activated NF-κB significantly. This result is similar to that of our previous studies with macrophages or lymphocytes, which revealed cag-independent NF-κB activation via TLR4 (27). Thus, it seems likely that the cag PAI system is required specifically for NF-κB activation in cells that are basically unresponsive to bacterial LPS.

Previously, several reports analyzed the signaling molecules involved in H. pylori-mediated NF-κB activation. These contained IKK complex (15, 16), TRAFs (16), NF-κB-inducing kinase (NIK) (15, 16), p21-activated kinase 1 (15), and Nod1 (24). Most experiments were based on overexpression of dominant-negative molecules, which sometimes leads to nonphysiological suppression of cell signaling. For example, dominant-negative NIK has been reported to suppress NF-κB activation induced by IL-1 and TNF-α (43). However, NIK knockout cells displayed normal NF-κB activation by IL-1 and TNF-α (44), indicating that the effect of dominant-negative NIK may not be physiological. Generally, knockout mice or cells derived from knockout mice are good tools for the evaluation of signaling pathways. However, in the H. pylori-infected C57BL/6 mouse model, we did not observe any difference between infection with wild-type (TN2) and cag mutant (TN2-ΔcagE) H. pylori strains, because both infected groups developed only mild gastritis (our unpublished observation). This scenario differs significantly from that of the Mongolian gerbil infection model, in which a variety of gastric diseases develops only when the animals are infected with cag-positive H. pylori (45, 46). We also found that MEFs are not useful for investigations of NF-κB activation mediated by H. pylori, because these cells are highly responsive to cag mutant H. pylori (Fig. 1).

Therefore, we used siRNA methods to examine the key molecules for pathogenesis in human epithelial cells. We found that siRNA for TAK1, TRAF6, and MyD88 significantly reduced cag PAI system required specifically for NF-κB activation in macrophage or lymphocytes. We also found that MEFs are not useful for investigations of NF-κB activation mediated by H. pylori, because these cells are highly responsive to cag mutant H. pylori (Fig. 1).

TLRs and IL-1Rs are known to recruit MyD88 to transduce the extracellular signal (35–37). Although there have been many reports of the role of TLRs in H. pylori infection, this topic remains controversial (47). In our experiments, we could not find any involvement of TLRs or IL-1Rs. These results are consistent with our previous report in which we excluded the major role for TLRs in epithelial cells (27). Therefore, we propose another mechanism of NF-κB activation via MyD88. In this study, siRNAs for IRAK1 and IRAK4 had no effect on H. pylori-induced NF-κB activation. Furthermore, we observed degradation of IRAK1 by H. pylori infection only in MEFs (data not shown), not in human epithelial cells (Fig. 6A). Because IRAK1 degradation or IRAK4 kinase activity is required for IL-1R- or TLR4-mediated cell signaling (48–51), our observation that H. pylori activates NF-κB without requiring IRAKs suggests a mode of MyD88 activation different from the receptor-mediated mechanism. The fact that cag PAI is a bacterial molecular transporter may support this idea. Thus, we speculate that H. pylori protein is translocated into epithelial cells via cag PAI to bind with MyD88, leading to subsequent activation of NF-κB in a receptor-independent manner. Because we could not find direct interaction between MyD88 and CagA protein, a reported substrate for the cag PAI secretion system, other bacterial molecules responsible for this phenomenon should be examined in future studies.

We also examined the role of Nod1, because Viala et al. (24) have reported that peptidoglycan from cag PAI-positive strains activates this intracellular protein. However, we could not find any
In conclusion, we have revealed that epithelial cells are different from other cell types with respect to responses to wild-type and cag mutant H. pylori. Epithelial cells activate NF-κB signaling via MyD88-TRAF6 and NF-κB signaling pathways of epithelial cells. This epithelial defense system may protect the host from virulent H. pylori invasion of the interstitial space by inducing proinflammatory cytokines, and it may protect the epithelial cell itself from cell death by inducing antiapoptotic genes. These responses result in gastritis, and when hyperactivated, they may lead to malignant transformation.

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