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Abstract: Inflammasome activation and caspase-1-dependent (CASP1-dependent) processing and secretion of IL-1 and IL-18 are critical events at the interface of the bacterial pathogen Helicobacter pylori with its host. Whereas IL-1 promotes Th1 and Th17 responses and gastric immunopathology, IL-18 is required for T reg differentiation, H. pylori persistence, and protection against allergic asthma, which is a hallmark of H. pylori-infected mice and humans. Here, we show that inflammasome activation in DCs requires the cytoplasmic sensor NLRP3 as well as induction of TLR2 signaling by H. pylori. Screening of an H. pylori transposon mutant library revealed that pro-IL-1 expression is induced by LPS from H. pylori, while the urease B subunit (UreB) is required for NLRP3 inflammasome licensing. UreB activates the TLR2-dependent expression of NLRP3, which represents a rate-limiting step in NLRP3 inflammasome assembly. ureB-deficient H. pylori mutants were defective for CASP1 activation in murine bone marrow-derived DCs, splenic DCs, and human blood-derived DCs. Despite colonizing the murine stomach, ureB mutants failed to induce IL-1 and IL-18 secretion and to promote T reg responses. Unlike WT H. pylori, ureB mutants were incapable of conferring protection against allergen-induced asthma in murine models. Together, these results indicate that the TLR2/NLRP3/CASP1/IL-18 axis is critical to H. pylori-specific immune regulation.
Brief report

Helicobacter urease-induced activation of the TLR2/NLRP3/IL-18 axis protects against asthma

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Inflammasome activation and caspase-1-dependent (CASP1-dependent) processing and secretion of IL-1β and IL-18 are critical events at the interface of the bacterial pathogen Helicobacter pylori with its host. Whereas IL-1β promotes Th1 and Th17 responses and gastric immunopathology, IL-18 is required for Treg differentiation. H. pylori persistence, and protection against allergic asthma, which is a hallmark of H. pylori–infected mice and humans. Here, we show that inflammasome activation in DCs requires the cytoplasmic sensor NLRP3 as well as induction of TLR2 signaling by H. pylori. Screening of an H. pylori transposon mutant library revealed that pro–IL-1β expression is induced by LPS from H. pylori, while the urease B subunit (UreB) is required for NLRP3 inflammasome licensing. UreB activates the TLR2-dependent expression of NLRP3, which represents a rate-limiting step in NLRP3 inflammasome assembly. ureB-deficient H. pylori mutants were defective for CASP1 activation in murine bone marrow–derived DCs, splenic DCs, and human blood-derived DCs. Despite colonizing the murine stomach, ureB mutants failed to induce IL-1β and IL-18 secretion and to promote Treg responses. Unlike WT H. pylori, ureB mutants were incapable of conferring protection against allergen-induced asthma in murine models. Together, these results indicate that the TLR2/NLRP3/CASP1/IL-18 axis is critical to H. pylori–specific immune regulation.

Introduction

Persistent infection of the gastric mucosa with H. pylori causes gastritis (1) and represents a major risk factor for the development of gastric cancer (2) but has also been inversely linked to the risk of allergic and chronic inflammatory diseases (3, 4). The outcome of the H. pylori/host interaction is determined both by host and bacterial genetic factors (5) as well as the infected individual’s preexposure profile (6). Treg-predominant responses are particularly pronounced in children (7) and can be recapitulated in experimental models of neonatal H. pylori infection (8), in which they are required for protection against allergen-induced asthma, i.e., a hallmark of H. pylori–infected mice and humans. Here, we show that inflammasome activation and cytokine processing as well as Treg differentiation and asthma protection is a consequence of H. pylori exerts proinflammatory effects that have both proinflammatory and antiinflammatory consequences that are differentially mediated by its β2 urease enzyme in promoting the activation of Gram-negative pathogens, i.e., TLR4, TLR5, and TLR9 or the IL-1 receptor, were not required for IL-1β secretion (Figure 1, E-G). Other surface and endosomal TLRs known to contribute to innate immune recognition of Gram-negative pathogens, i.e., TLR4, TLR5, and TLR9 or the IL-1 receptor, were not required for IL-1β secretion (Figure 1, E-G). Other surface and endosomal TLRs known to contribute to innate immune recognition of Gram-negative pathogens, i.e., TLR4, TLR5, and TLR9 or the IL-1 receptor, were not required for IL-1β secretion (Figure 1, E-G).
lower levels and exhibited higher gastric mucosal IFN-γ expression (Figure 1, J and K), which correlates well with higher frequencies of IFN-γ-expressing CD4+ T cells in the mesenteric lymph nodes (MLNs) of Tlr2–/– mice relative to those in WT mice (Supplemental Figure 2T). The combined results suggest that H. pylori activates the inflammasome in a TLR2- and NLRP3-dependent manner and benefits from this process because it promotes H. pylori persistence.

Genome-wide screening for factors involved in IL-1β secretion reveals a role for H. pylori LPS and urease. Known activators of the NLRP3 inflammasome include both foreign and endogenous compounds, with the best-understood being urate crystals, asbestos, ATP, and bacterial pore-forming toxins (15). We were able to exclude a role for the H. pylori immunomodulator γ-glutamyltranspeptidase GGT and the Cag pathogenicity island in CASP1 activation and IL-1β secretion (Supplemental Figure 3, A and B). To search for H. pylori factors involved in inflammasome activation in a genome-wide manner, we took advantage of a previously described tn mutant library (16). As IL-1β secretion by H. pylori-
B and C). IL-1β expression upon stimulation with both types of LPS was MyD88- and TLR4-dependent (Supplemental Figure 4D). Remarkably, of the 8 tn insertions mapping to the urease gene cluster, all affected 2 genes encoding the structural urease subunits, ureA or ureB (Supplemental Table 1). A gene-specific deletion mutant lacking both UreA and UreB proteins (G27 Δure, Supplemental Figure 5A) phenocopied the effect of the tn insertion mutants, which could be attributed to its failure to activate CASP1 (Figure 2, C and D). In contrast, il1b transcription was normal (Figure 2E). Coculturing of murine splenic CD11c+ DCs and human blood-derived DCs confirmed the defect of the H. pylori Δure and Δ146 mutants with respect to IL-1β secretion; in contrast, the secretion of IL-18, which does not require transcriptional activation, was almost at WT levels in the case of the Δ146 mutant (Figure 2, F–H). In summary, our screen identified H. pylori factors regulating CASP1-dependent cytokine secretion at two distinct levels, one transcriptional and one posttranslational.
no effect on the two processes (Figure 2M and data not shown). Taken together, the results suggest that UreB signals via TLR2 to prime NLRP3 expression, which appears to be a rate-limiting step in 

*H. pylori*–induced inflammasome activation and IL-1β processing. This is particularly interesting because TLR2 has not yet been described to be activated by bacterial (non-lipo-) proteins.

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To investigate the consequences of LPS and urease deficiency in vivo, Δure and Δ146 mutants were generated in the mouse-colonizing strains PMSS1 and/or SS1 and used for experimental infections of adult or neonatal C57BL/6 mice. Whereas Δ146 failed to colonize under all circumstances (data not shown), the PMSS1 Δure mutant colonized adult infected mice at WT levels for at least 3 months, without evidence of having regained urease expression (Supplemental Figure 6, A and B). In contrast, SS1 Δure consistently failed to colonize (data not shown).

Given the similarities in outcome of TLR2 deficiency of the host on the one hand and urease deficiency of the bacteria on the other (i.e., lack of CASP1 activation), we hypothesized that *H. pylori* urease might provide a TLR2-mediated signal to promote inflammasome/CASP1 activation. As NLRP3 expression can be primed by TLR signaling, we asked whether NLRP3 transcript and protein levels in BMDCs are affected by *H. pylori* exposure. Indeed, WT *H. pylori* infection efficiently induced NLRP3, but not AIM2 or NLRC4, expression at the transcript and protein levels in a TLR2-, MyD88- and NF-κB–dependent manner; this was not observed in BMDCs infected with Δure *H. pylori* (Figure 2, I and J; Supplemental Figure 5, B–H; and data not shown). Interestingly, the defect of the Δure mutant with respect to CASP1 activation and IL-1β secretion could be rescued by recombinant UreB, but not UreA or the GST tag control; this effect was only seen in WT, but not in Tlr2−/−, BMDCs (Figure 2, K–M). Heat-inactivated UreB had no effect on the two processes (Figure 2M and data not shown). Taken together, the results suggest that UreB signals via TLR2 to prime NLRP3 expression, which appears to be a rate-limiting step in *H. pylori*–induced inflammasome activation and IL-1β processing. This is particularly interesting because TLR2 has not yet been described to be activated by bacterial (non-lipo-) proteins.

*H. pylori* urease is required for CASP1 activation, Treg responses, and asthma protection in vivo. To investigate the consequences of LPS and urease deficiency in vivo, Δure and Δ146 mutants were generated in the mouse-colonizing strains PMSS1 and/or SS1 and used for experimental infections of adult or neonatal C57BL/6 mice. Whereas Δ146 failed to colonize under all circumstances (data not shown), the PMSS1 Δure mutant colonized adult infected mice at WT levels for at least 3 months, without evidence of having regained urease expression (Supplemental Figure 6, A and B). In contrast, SS1 Δure consistently failed to colonize (data not shown).
not shown), confirming that urease proficiency is required for mouse colonization in certain strain backgrounds (17). Interestingly, PMSS1Δure induced significantly less gastric production of IL-1β and IL-18 and less active CASP1 than the parental WT strain (Supplemental Figure 6, C–E). In line with the increased gastric IFN-γ expression of Tlr2Δ/+ and Nlrp3Δ/Δ animals (Figure 1K), Δure-infected mice exhibited higher gastric mucosal IFN-γ expression and more IFN-γ+CD4+ cells in the MLNs than infected WT animals (Supplemental Figure 6, F and G). A similar pattern was observed in neonatally infected animals, in which CASP1 activation, NLRP3 expression, and IL-18 secretion were also found to depend on urease proficiency of H. pylori; moreover, colonization levels of the Δure mutant were strongly reduced relative to the WT strain in neonatally infected mice (Figure 3, A–D). As in adult infected mice, the Δure mutant elicited higher Ifng expression (Figure 3E). Interestingly, H. pylori urease was further required for the efficient protection against allergen-induced asthma that is a hallmark of neonatally infected mice. All examined parameters of ovalbumin-induced allergic asthma, i.e., bronchoalveolar eosinophilia, lung inflammation, and goblet cell metaplasia as well as pulmonary Th2 cytokine production, were clearly reduced in infected WT animals but not in Δure-infected animals (Figure 3, F–I, and Supplemental Figure 7A). Similar results were obtained in the house dust mite model of allergic asthma (Figure 3, J–M). Moreover, protection was abrogated by a blocking antibody targeting IL-18 and in Tlr2Δ/+ mice (Figure 3, J–M), which, similar to Nlrp3Δ/+ mice, had a lower bacterial burden than WT mice (Figure 3N). Protection against ovalbumin-induced asthma could be adoptively transferred via immunomagnetically purified CD25+ Tregs from H. pylori infected WT donors but not Δure-infected donors; Tregs from H. pylori infected WT Tlr2Δ/+ and Nlrp3Δ/+ animals also failed to confer protection (Supplemental Figure 7, B and C). The quantification of CD25 FoxP3+ Tregs in MLNs further revealed lower Treg frequencies in Δure-infected mice relative to infected WT mice and Tlr2Δ/+ mice relative to WT mice (Supplemental Figure 7, D and E). In summary, the findings described here document a previously unrecognized role of H. pylori urease in innate immune recognition and H. pylori persistence that presumably is unrelated to its function in acid resistance. Here, we show that UreB promotes the TLR2-dependent expression of NLRP3, a critical component of the inflammasome that is required for CASP1 activation and IL-1β/IL-18 processing (see model in Supplementary Figure 8). Infection with urease gene deletion mutants phenocopies the effects of TLR2 and NLRP3 deficiency. The combined results confirm a critical contribution of the TLR2/NLRP3/CASP1/IL-18 axis to microbially induced immune regulation and introduce the H. pylori urease as a novel immunomodulator of this important human pathobiont.

Methods

**Animal experimentation.** C57BL/6 WT, Casp1Δ/Δ, Tlr2Δ/+ , Tlr4Δ/+ , Tlr5Δ/+ , Tlr6Δ/+ , Myd88Δ/+ , TrifΔ/Δ, Nlrp3Δ/Δ, IfnarΔ/Δ, IfnγΔ/Δ, NoD2Δ/+ , P2x7Δ/Δ, and Aim2Δ/+ mice were originally obtained from Charles River Laboratories. Nlr4Δ/+ and AscΔ/+ mice were provided by Genentech. Nlrp6Δ/+ mice were provided by Millenium Pharmaceuticals. Ilr1Δ/+ mice were provided by Manfred Kopf. Mice were infected orally with 10^9 CFU H. pylori PMSS1 at 6 weeks or 7 days of age. Bacterial colonization was assessed by colony counting. The procedures used for asthma induction and cytokine quantification by qPCR, ELISA, and FACS are described in the Supplemental Methods.

**H. pylori strains, infection of DCs, and tn library screening.** H. pylori strains and culture conditions as well as the procedures used for the differentiation and immunomagnetic isolation of DCs and for tn library screening are described in the Supplemental Methods, along with protocols for Western blotting and purification of recombinant proteins.

**Statistics.** GraphPad Prism (GraphPad Software) was used for statistical analyses. All P values were calculated by Mann-Whitney U test.

**Study approval.** All animal experimentation was reviewed and approved by the Veterinary Office of the canton of Zurich (Zurich, Switzerland) (licenses 24/2013 and 170/2014 to A. Müller).

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