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Caspase-1 Has Both Proinflammatory and Regulatory Properties in *Helicobacter* Infections, Which Are Differentially Mediated by Its Substrates IL-1β and IL-18

Iris Hitzler,* Ayca Sayi,* Esther Kohler,* Daniela B. Engler,* Katrin N. Koch,* Wolf-Dietrich Hardt,† and Anne Müller*

The proinflammatory cysteine protease caspase-1 is autocatalytically activated upon cytosolic sensing of a variety of pathogen-associated molecular patterns by Nod-like receptors. Active caspase-1 processes pro–IL-1β and pro–IL-18 to generate the bioactive cytokines and to initiate pathogen-specific immune responses. Little information is available on caspase-1 and inflammasome activation during infection with the gastric bacterial pathogen *Helicobacter pylori*. In this study, we addressed a possible role for caspase-1 and its cytokine substrates in the spontaneous and vaccine-induced control of *Helicobacter* infection, as well as the development of gastritis and gastric cancer precursor lesions, using a variety of experimental infection, vaccine-induced protection, and gastric disease models. We show that caspase-1 is activated and IL-1β and IL-18 are processed in vitro and in vivo as a consequence of *Helicobacter* infection. Caspase-1 activation and IL-1 signaling are absolutely required for the efficient control of *Helicobacter* infection in vaccinated mice. IL-1R−/− mice fail to develop protective immunity but are protected against *Helicobacter*-associated gastritis and gastric preneoplasia as a result of their inability to generate *Helicobacter*-specific Th1 and Th17 responses. In contrast, IL-18 is dispensable for vaccine-induced protective immunity but essential for preventing excessive T cell-driven immunopathology. IL-18−/− animals develop strongly accelerated pathology that is accompanied by unrestricted Th17 responses. In conclusion, we show in this study that the processing and release of a regulatory caspase-1 substrate, IL-18, counteracts the proinflammatory activities of another caspase-1 substrate, IL-1β, thereby balancing control of the infection with the prevention of excessive gastric immunopathology. *The Journal of Immunology*, 2012, 188: 3594–3602.


cells of the innate immune system recognize microbial pathogens through their conserved molecular structures, termed pathogen-associated molecular patterns (PAMPs). Examples of PAMPs include LPS, lipoproteins, flagellins, peptidoglycan, and microbial nucleic acids (1). PAMPs are recognized by extracellular or endosomal, membrane-bound TLRs or by cytosolic Nod-like receptors (NLRs) (2, 3). Among the various NLRs, Nod1 and Nod2 recognize peptidoglycan metabolites and induce the transcription factor NF-κB to activate innate and adaptive immune response genes (4); in contrast, most other NLRs are involved in the assembly of multiprotein complexes called inflammasomes, which activate the cysteine protease caspase-1 (5). The interaction between the NLRs and caspase-1 in inflammasomes is mediated by the bipartite adaptor protein ASC (6). Autocatalytically activated caspase-1 processes the cytoplasmic precursors of IL-1β and IL-18 to generate the mature, biologically active cytokines, which are subsequently released to initiate inflammation and defense mechanisms (2, 7). Activation of inflammasomes is critical for the recognition of numerous Gram-negative and Gram-positive bacteria, including *Legionella pneumophila* (8), *Shigella flexneri* (9), *Pseudomonas aeroginosa* (10), *Bordetella pertussis* (11), *Salmonella enterica* (12), *Staphylococcus aureus* (12)*,*, and *Listeria monocytogenes* (13), as well as for the detection and response to influenza viruses (14) and alulm adjuvants (15).

Several distinct inflammasomes can be distinguished based on the NLR involved. Activation of the NLR4 inflammasome is triggered by cytosolic delivery of flagellin via type III or type IV secretion systems of Gram-negative bacteria, such as *Salmonella typhimurium*, *P. aeruginosa*, or *L. pneumophila* (3, 7). The NLRP3 inflammasome is activated by both foreign and endogenous molecules that include urate crystals, ATP, bacterial pore-forming toxins, and particulate matter, such as asbestos and silica (2, 3). The signals recognized by the NLRP1 inflammasome are less well understood, but they seem to include anthrax lethal toxin (16).

Persistent gastric infection with the Gram-negative bacterial pathogen *Helicobacter pylori* results in chronic gastritis (17) and predisposes carriers to a high risk for developing gastric and duodenal ulcers, gastric cancer, and gastric MALT lymphoma (18–20). We showed recently that the CD4+ T cell-mediated immune response to the pathogen, rather than the presence of the bacteria per se, is a driving force behind the infection-associated gastric preneoplastic immunopathology manifesting as atrophic gastritis, epithelial hyperplasia, and intestinal metaplasia (21). The caspase-1 substrate IL-1β is highly induced in the gastric mucosa of *H. pylori*-infected individuals (22), and polymorphisms associated with increased steady-state levels of IL-1β predispose carriers to
gastric cancer (23). The stomach-specific expression of human IL-1β is sufficient to induce gastric inflammation and gastric cancer in transgenic mice (24).

Surprisingly, little is known about the mechanisms leading to inflammasicone activation by H. pylori or the NLRs involved in the process. H. pylori peptidoglycan appears to be detected by the NLR Nod1 in a type IV secretion system-dependent manner (25); however, although H. pylori-activated Nod1 triggers the activation of the NF-xB and ISGF3-signaling pathways (25, 26), it is not known to activate caspase-1. In this study, we determined the role of caspase-1 and its cytokine substrates in the control and pathogenesis of Helicobacter infection using several complementary Helicobacter-induced gastric disease and colonization models. Our findings reveal dual functions for caspase-1 in H. pylori infection that are mediated by active IL-1β and IL-18. Although caspase-1–mediated IL-1β processing is absolutely required for the efficient control of H. pylori infection in vaccinated mice and for the induction of Helicobacter-associated gastric immunopathology, active IL-18 balances excessive anti-Helicobacter T cell responses and prevents gastric immunopathology by restricting pathogenic Th17 responses. Thus, caspase-1 exhibits both proinflammatory and regulatory properties in the context of Helicobacter infection that are exerted through its two cytokine substrates.

Materials and Methods

Animal experimentation

C57BL/6 wild-type, caspase 1−/−, IL-1R−/−, and IL-18−/− mice were originally purchased from Charles River Laboratories (Sulzfeld, Germany). All mice were bred at a University of Zurich specific pathogen-free facility. Mice were housed in individually ventilated cages, and mixed-gender groups were included in studies at 5–6 wk of age (gender differences were not observed in the strains and infection models used). All animal experimentation was reviewed and approved by the Zurich cantonal veterinary office. For immunization, mice received 500 μg H. pylori SS1 whole-cell sonicate along with 10 μg cholera toxin (CT; List Biologicals, Campbell, CA) by oral gavage in a total volume of 200 μl three times at weekly intervals. Two weeks after the last immunization, immunized and naive control mice were infected with 105 CFU H. pylori SS1 (27), grown as described previously (21). Mice were sacrificed 2 wk after challenge.

Bacterial colonization was assessed by colony count assay, as described earlier (21), to determine CFU/stomach. For the purpose of Helicobacter felis infection, mice were infected with two consecutive orogastric doses of ~106 CFU H. felis CS1 (ATCC 49179), grown as described (21). At study end points, stomachs were retrieved and dissected longitudinally into several equally sized pieces. For the quantitative PCR-based assessment of H. felis colonization, genomic DNA-based assessment of caspase-1 activation, BMDCs were infected for 16 h with H. pylori strain SS1, the recently described parental strain of SS1, PMSS1 (28), or H. felis at a multiplicity of infection ~50. BMDCs were pretreated for 3 h with 5 ng/ml LPS to stimulate IL-1β expression, where indicated. Infected BMDCs or MLN preparations were stained with a carboxyfluorescein-labeled caspase-1–specific FLICA inhibitor (FAM-YVAD-FMK; Immunoculture Technologies, Bloomington, MN), according to the manufacturer’s instructions, and analyzed by flow cytometry. Dead cells were excluded by gating out propidium iodide-positive cells. To detect the full-length and autocratically activated caspase-1, cell extracts and TCA-precipitated supernatants were subjected to Western blotting with an activated caspase-1–specific Ab (anti-mouse Casp1 p10, sc514; Santa Cruz).

Preparation of gastric single-cell suspensions and flow cytometry

One sixth of every stomach (antrum and corpus) was digested in 1 mg/ml collagenase for 45 min at 37°C, with shaking prior to mechanical disruption between glass slides and filtering through a cell strainer (40 μm). Single-cell suspensions were not observed in the strains and infection models used. All mice were bred at a University of Zurich specific pathogen-free facility. Mice were housed in individually ventilated cages, and mixed-gender groups were included in studies at 5–6 wk of age (gender differences were not observed in the strains and infection models used). All animal experimentation was reviewed and approved by the Zurich cantonal veterinary office. For immunization, mice received 500 μg H. pylori SS1 whole-cell sonicate along with 10 μg cholera toxin (CT; List Biologicals, Campbell, CA) by oral gavage in a total volume of 200 μl three times at weekly intervals. Two weeks after the last immunization, immunized and naive control mice were infected with 105 CFU H. pylori SS1 (27), grown as described previously (21). Mice were sacrificed 2 wk after challenge.

Statistical analysis

GraphPad Prism (GraphPad Software, La Jolla, CA) was used for statistical analyses. All p values were calculated by Mann–Whitney U test. In all scatter plot graphs, the medians are indicated by horizontal bars. In bar graphs, the SEM is indicated by vertical bars.

Results

H. pylori activates caspase-1 and induces mature IL-1β and IL-18 secretion in vitro and in vivo

To test whether H. pylori activates caspase-1 in vitro, we generated BMDCs, infected them overnight with H. pylori strain SS1, and quantified activated caspase-1 using the carboxyfluorescein-labeled caspase-1 substrate FAM-YVAD-FMK (FLICA). A significantly higher proportion of infected cells compared with uninfected cells showed evidence of caspase-1 activation under these conditions; the physical separation of the bacteria and their host cells by a Transwell filter abrogated caspase-1 activation (Fig. 1A). The activation of caspase-1 by H. pylori SS1 was further confirmed by detection of the autocratically activated 10-kDa subunit of caspase-1 by Western blotting; it was observed upon BMDC infection with SS1, as well as with the recently described parental strain of SS1, H. pylori PMSS1 (28), and with the closely related species H. felis (Supplemental Fig. 1A). Similarly, mice that had been infected experimentally with H. pylori SS1 for 2 wk exhibited higher numbers of FLICA+ cells in their MLNs (Fig. 1B), the sites of H. pylori–specific T cell priming (29, 30).

Caspase-1 cleaves the precursor molecules of IL-1β and IL-18 to generate the mature, bioactive forms of the two cytokines. To first
assess whether *H. pylori* infection induces pro–IL-1β and/or pro–IL-18 expression in BMDCs in vitro, we quantified the transcript levels of both cytokines. Coculture of BMDCs with *H. pylori* induced the expression of pro–IL-1β, but not pro–IL-18, transcripts (Fig. 1C). However, the release of both mature cytokines into the culture supernatant was induced by *H. pylori* infection under these conditions (Fig. 1D), arguing that IL-1β, but not IL-1β, precursor molecules are preformed and stored in BMDCs rather than synthesized de novo upon infection. The secretion of mature IL-1β and IL-18 into the supernatant was induced by *H. pylori* infection, as well as by PMSS1 and *H. felis* upon coculture with BMDCs, and was entirely dependent on caspase-1 expression (Supplemental Fig. 1B, 1C). BMDCs generated from caspase-1–/– bone marrow were incapable of processing IL-1β or IL-18 upon infection (Supplemental Fig. 1B, 1C). Preincubation of the BMDCs with *Escherichia coli* LPS strongly increased the production of mature IL-1β, but not of IL-18, by infected BMDCs (compare absolute values in Fig. 1D and Supplemental Fig. 1B, 1C).

Analogous to the in vitro situation, the differential induction of pro–IL-1β, but not of pro–IL-18, transcripts could be detected in the gastric mucosa of *H. pylori*-infected mice (Fig. 1E), despite the fact that the mature forms of both cytokines are highly upregulated in the tissue at the protein level (Fig. 1F). Prior immunization of mice with a *Helicobacter* whole-cell vaccine adjuvanted with CT further exacerbates the gastric production of both mature cytokines (Fig. 1E, 1F). Taken together, the results suggest that caspase-1 is activated during *Helicobacter* infection in vitro and in vivo, leading to the processing of cytokine precursors and the accumulation of mature IL-1β and IL-18 at the site of infection.

**Caspase-1 is required for vaccine-induced protective immunity against *H. pylori* infection and at the same time controls excessive gastric immunopathology**

Having observed that *H. pylori* infection efficiently activates caspase-1 in vitro and in vivo, we sought to elucidate the effects of caspase-1 gene deletion in mouse models of vaccine-induced protective immunity and in experimental infection models. We orally immunized wild-type and caspase-1–/– mice with three consecutive weekly doses of an *H. pylori* whole-cell sonicate vaccine adjuvanted by CT and determined the outcome of autologous challenge infection in the vaccinated mice compared with naive mice of both strains. Immunized caspase-1–/– mice were significantly less able to control a challenge infection than were wild-type controls (Fig. 2A) and also had lower levels of gastric leukocyte and CD4+ T cell infiltration (Fig. 2B, 2C), two parameters known to represent independent correlates of vaccine-induced protection (31). Surprisingly, the gastric expression of IFN-γ and IL-17 was normal in the vaccinated caspase-1–/– animals relative to their wild-type counterparts (Fig. 2D, 2E). Despite their apparent defect in developing protective immunity upon vaccination, naive (unvaccinated) caspase-1–/– mice controlled the experimental infection more effectively than did their naive wild-type counterparts, being colonized by one order of magnitude less densely (Fig. 2A). The lower bacterial burden of naive caspase-1–/– mice correlated with their strongly elevated levels of gastric IL-17 (Fig. 2E), which differed little from that of their vaccinated littermates.

To further elucidate the outcome of experimental infection in a *Helicobacter*-dependent gastric disease model, caspase-1–/– and wild-type mice were infected for 1 or 3 mo with the close *H. pylori* relative *H. felis*, a strain that is known to colonize mice persistently and to efficiently induce chronic gastritis and gastric preneoplastic pathology (21, 32). In this model, caspase-1–/– mice were also colonized at lower levels than were wild-type controls (as observed with the vaccine strain, SS1; Fig. 2A), both at 1 mo (Fig. 3A) and 3 mo postinfection (Supplemental Fig. 2), indicating that caspase-1–/– mice were able to control the infection more efficiently. As a consequence, the caspase-1–/– strain exhibited substantially more gastric inflammation and preneoplastic immunopathology, manifesting histologically as gastric atrophy, epithelial hyperplasia, and intestinal metaplasia, than did *H. pylori*–infected wild-type mice (Fig. 3B, 3C). Furthermore, caspase-1–/– mice were also significantly more able to control a challenge infection in the vaccinated mice compared with naive mice of both strains (Fig. 3D). It is noteworthy that, despite the significant reduction in gastric inflammation and preneoplastic pathology, caspase-1–/– mice still exhibited a significant increase in gastric leukocyte and CD4+ T cell infiltration (Fig. 3E, 3F), along with a marked increase in the serum levels of IL-1β and IL-18 (Fig. 3G, 3H), which correlated with the gastric expression of IFN-γ and IL-17 (Fig. 3I, 3J). These results indicate that caspase-1 controls gastric inflammation and preneoplastic pathology, and suggest that caspase-1 deficiency fails to control inflammation and preneoplastic pathology and may also contribute to the development of gastric inflammation and preneoplastic pathology, and suggest that caspase-1 deficiency may also play a role in the development of gastric inflammation and preneoplastic pathology.

**FIGURE 1.** Caspase-1 activation and IL-1β/IL-18 secretion are induced by *H. pylori* infection in vitro and in vivo. Percentages of *H. pylori*-infected (strain SS1, multiplicity of infection of 50, 16-h infection) and uninfected BMDCs (A; data pooled from three independent experiments) and total MLN cells isolated from seven infected and seven uninfected donor mice (B) that are positive for activated caspase-1, as determined by FLICA staining. Horizontal lines represent median values; BMDCs were separated from the bacteria by Transwell filters where indicated. The BMDCs infected with *H. pylori*, as described in (A), were further analyzed with respect to the expression of IL-1β and IL-18 at the RNA level (C) and protein level (D) by real-time PCR (normalized to GAPDH) and ELISA of culture supernatants. IL-1β and IL-18 expression in the gastric mucosa of immunized and infected (imm/inf; n = 11 mice), infected (inf; n = 12), or uninfected mice (uninf; n = 8), as determined by real-time RT-PCR (E, normalized to GAPDH) and ELISA of gastric mucosal extract (F). Data are shown as mean ± SEM.
wild-type mice at 1 mo (Fig. 3B, 3C) and 3 mo postinfection (Supplemental Fig. 2). The lower bacterial burden and increased immunopathology of the caspase-1−/− strain coincided with higher gastric expression of IL-17, but not of IFN-γ, relative to wild-type mice (Fig. 3D, 3E). In summary, the results suggest that caspase-1 plays a dual role in the context of H. pylori infection. On one hand, its activity is required for vaccine-induced protective immunity against H. pylori; on the other hand, caspase-1 prevents excessive immune activation against H. pylori infection in naive mice and, thereby, restricts infection-associated gastric immunopathology.

The caspase-1 substrate IL-1, but not IL-18, is required for vaccine-induced protective immunity to H. pylori. Caspase-1 cleaves the precursor molecules of IL-1β and IL-18 to generate the mature, bioactive forms of the two cytokines (33). To determine whether the postulated proinflammatory and regulatory activities of caspase-1 require IL-1β and/or IL-18, or neither of the two, we first investigated the phenotypes of genetically modified strains lacking either IL-1R or IL-18 in the vaccination/challenge model. Interestingly, although IL-1R−/− animals exhibited a clear defect in generating vaccine-induced protective immunity, IL-18−/− mice were protected as well as wild-type mice (Fig. 2A).

FIGURE 2. Lack of caspase-1 impairs vaccine-induced protective immunity against H. pylori infection. (A) H. pylori colonization levels as assessed by colony count assay (CFU) for immunized and challenged (imm/inf) and infected (inf) wild-type and caspase-1−/− mice. Horizontal lines represent median values; each symbol represents one mouse. Percentages, with regard to total stomach cells, of leukocytes (CD45+) (B) and CD4+ T cells (C) infiltrating the gastric mucosa in immunized and challenged (imm/inf) and infected (inf) wild-type and caspase-1−/− mice (subsets of the mice shown in (A)); horizontal lines represent the medians. Real-time RT-PCR results for IFN-γ (D) and IL-17 (E) expression in the gastric mucosa of the mice shown in (A), normalized to GAPDH (mean ± SEM).

FIGURE 3. Lack of caspase-1 improves spontaneous control of Helicobacter infection and aggravates gastric immunopathology. (A) Colonization levels, as determined by quantitative PCR of the H. felis flaB gene, of wild-type and caspase-1−/− mice infected with H. felis for 1 mo. Medians are indicated by horizontal lines; each symbol represents one mouse. (B) Histopathology scores assigned to every mouse shown in (A) for all indicated parameters on a scale of 0–6. (C) Representative micrographs of Giemsa-stained sections of one H. felis-infected wild-type (left panel) and caspase-1−/− (right panel) mouse. Scale bar, 50 μm. Real-time RT-PCR results for gastric IFN-γ (D) and IL-17 (E) expression of the mice shown in (A) and (B), normalized to GAPDH.
animals (Fig. 4A). Several correlates of protection, including the gastric infiltration of CD45+ leukocytes, CD4+ T cells, and c-Kit+ mast cells, confirmed the defect of the IL-1R-/- strain, but not of the IL-18-/- strain, in generating protective immunity (Fig. 4B–D). The complete lack of protection in IL-1R-/- animals was reflected in their strongly reduced gastric IFN-$$\gamma$$ and IL-17 expression levels (Fig. 4E, 4F). In contrast, the well-protected IL-18-/- mice exhibited almost normal gastric expression of IL-17 and a relatively modest defect in IFN-$$\gamma$$ expression (Fig. 4E, 4F).

Because control of the experimental infection in naive (as opposed to vaccinated) caspase-1-/- mice was more efficient than in the wild-type animals (Figs. 2A, 3A), we also examined and compared the colonization levels in naive mice of the IL-1R-/- and IL-18-/- backgrounds with wild-type controls. Interestingly, although the IL-1R-/- strain was unable to spontaneously reduce the colonization levels compared with wild-type mice, the IL-18-/- animals phenocopied the caspase-1-/- strain (Fig. 4A). As observed for the caspase-1-/- mice, the spontaneous reduction of the challenge infection in naive IL-18-/- mice correlated with strongly enhanced gastric expression of IL-17 (Fig. 4F). Taken together, the results suggest that caspase-1 and its substrate IL-1$$\beta$$, but not IL-1$$\beta$$ signaling, is required for protective, Th1/Th17-driven immunity against H. pylori that is conferred by a whole-cell sonicate vaccine. Conversely, the spontaneous control of the challenge infection in naive caspase-1-/- and IL-18-/- mice suggests that caspase-1 has both proinflammatory and immunosuppressive properties that are differentially mediated by its two alternative substrates, IL-1$$\beta$$ and IL-18.

**FIGURE 4.** Vaccine-induced protective immunity to H. pylori depends on the caspase-1 substrate IL-1$$\beta$$ but not IL-18. (A) H. pylori colonization levels, as assessed by colony count assay, for immunized and challenged versus infected-only wild-type, IL-1R-/-, and IL-18-/- mice. Each symbol represents one mouse. Percentages, with regard to total stomach cells, of leukocytes (CD45+)(B), CD4+ T cells (C), and mast cells (c-Kit+) (D) infiltrating the gastric mucosa of the mice shown in (A). Real-time RT-PCR results for gastric IFN-$$\gamma$$ (E) and IL-17 (F) expression of the mice shown in (A), normalized to GAPDH.
not control *H. felis* colonization levels better than wild-type animals (Fig. 5C), and did not express more gastric IL-17 and IFN-γ (Fig. 5D, 5E). In summary, the cytokines IL-1 and IL-18 fulfill distinct functions in adaptive immune activation, with IL-1 promoting immunity and IL-18 counteracting excessive immunopathology.

IL-1 signaling is required for the spontaneous control of *Helicobacter* infection and for the induction of preneoplastic pathology

To assess a possible role for IL-1 signaling in the control of *Helicobacter* infection and in the induction of infection-associated gastric pathology, wild-type and IL-1R<sup>2/2</sup> mice were infected with *H. felis* for 3 mo. Interestingly, IL-1R<sup>2/2</sup> mice were clearly protected against the gastritis and gastric preneoplasia that arises in wild-type mice after 3 mo of infection with *H. felis* (Fig. 6A, 6B). The resistance of IL-1R<sup>2/2</sup> animals to gastric pathology correlated well with their higher bacterial loads (Fig. 6C) and their lower gastric expression of IL-17 and IFN-γ (Fig. 6D, 6E). In conclusion, the results from the *H. felis*-induced disease model imply that the regulatory properties of caspase-1 (which restrict the spontaneous control of *H. pylori* and *H. felis* infection and limit gastric immunopathology) depend on the processing of IL-18, whereas the proinflammatory/pathogenic properties of caspase-1 are mediated via IL-1. Taken together, the combined results from the vaccination and disease models strongly imply that caspase-1 has both proinflammatory and regulatory activities, and these depend differentially on its two alternative substrates, IL-1β and IL-18 (Fig. 7).

**Discussion**

Autocatalytic caspase-1 activation by the inflammasome is essential for the control of numerous Gram-negative and Gram-positive bacteria (2, 3, 5). To our knowledge, we provide the first evidence that *H. pylori* infection activates caspase-1, leading to IL-1β/IL-18 processing and secretion, both in cultured dendritic cells (DCs) and in vivo. Our findings further imply that the two caspase-1 cytokine substrates unexpectedly have very different functions in shaping the adaptive immune response to *H. pylori* infection and may balance one another in promoting infection control and immunity on one hand, while preventing excessive
immunopathology on the other (see model in Fig. 7). Our results confirm and extend a previous study demonstrating a direct role for IL-1 signaling in the induction of gastritis and gastric carcinogenesis (24). In their report, Tu et al. (24) showed that the stomach-specific expression of human IL-1β was sufficient to induce gastric inflammation and gastric cancer in transgenic mice. In line with this observation, IL-1R−/− mice were completely protected against infection-induced gastritis and showed no evidence of preneoplastic pathology in our H. felis-induced disease model. We attribute the phenotype of IL-1R−/− mice to their inability to generate Th1- and Th17-polarized T cell responses to the infection, which is evident in experimental infection, as well as in vaccination/challenge models. Thus, our results support a crucial role for IL-1 signaling in the differentiation of Th1 and Th17 cell subsets, both of which are essential for the development of protective immunity on the one hand and gastric CD4+ T cell-driven immunopathology on the other (21, 31, 34, 35).

IL-18−/− mice showed the completely opposite phenotype. They developed normal protective immunity to H. pylori under conditions of vaccination followed by challenge infection, despite a somewhat reduced ability to generate Th1- and Th17-polarized effector T cell responses. These results are in line with a previous publication reporting normal vaccine-induced protective immunity in IL-18−/− mice (36), but they are incongruent with another report demonstrating a modest impairment of protection due to IL-18 gene deficiency (37) using a similar vaccination protocol. However, rather unexpectedly, naive (nonimmunized) IL-18−/− mice were capable of spontaneously controlling experimental infection with H. pylori SS1 or H. felis, thereby phenocopying caspase-1−/− animals and exhibiting striking similarity to mice in which regulatory T cells had been depleted quantitatively (32). The spontaneous control of the infection in IL-18−/− and caspase-1−/− mice coincided with their strongly elevated gastric IL-17 levels and severely enhanced and accelerated gastric preneoplastic pathology. However, it should be noted that our experimental model does not allow us to rule out the possibility that Helicobacter colonization levels are lower, not because the pathogen-specific Th17 responses are enhanced in IL-18−/− and caspase-1−/− mice but because unspecific inflammation (triggered by infection) generates a less hospitable niche. Nevertheless, we strongly favor the interpretation that the lack of IL-18 (in IL-18−/− mice) or of its processing (in caspase-1−/− mice) impairs Th1 responses on the one hand, as shown previously (38, 39), but enhances Th17 responses on the other, with the latter effects driving the Helicobacter-associated phenotypes of the respective gene-targeted strains.

Similar results were reported in a DSS-induced model of chronic intestinal inflammation, which also revealed an increased sensitivity of IL-18−/− mice compared with wild-type mice (40, 41). Both studies linked the lack of expression of NLRP3, caspase-1, and ASC in intestinal epithelial cells to colitis hypersusceptibility, which could be alleviated, at least partially, by administration of rIL-18 (40, 41). One study attributed the protective activity of IL-18 to its putative beneficial effects on enterocyte proliferation and maintenance of epithelial integrity (40). In our Helicobacter infection models, the enhanced mucosal IL-17 expression that characterizes infected IL-18−/− and caspase-1−/− mice suggests that IL-18 is required for the suppression of pathogenic Th17 responses, rather than the maintenance of epithelial integrity and homeostasis. Results from other colitis models are in conflict with...

**FIGURE 7.** Caspase-1 activation and IL-1β and IL-18 secretion govern adaptive immunity to Helicobacter. Schematic summarizing the effects of Helicobacter-induced caspase-1 activation on Th cell differentiation. Upon encounter of Helicobacter, DCs (shown here; other APCs may also be important) phagocytose the bacteria and present MHC class II-restricted Helicobacter Ags on their surface. In addition, as-yet-uncharacterized Helicobacter PAMPs activate TLRs or other pattern recognition receptors to induce transcription of the IL-1β gene; in contrast, pro–IL-1β is not transcriptionally induced but preformed and stored in naive DCs. Additional PAMPs are detected by cytoplasmic NLRs and activate procaspase-1, which is autocatalytically processed and cleaves pro–IL-18 and pro–IL-1β to generate the mature cytokines. Both cytokines must be produced for the efficient induction of Th1 differentiation of Helicobacter-specific T cells; IL-1β further promotes Th17 differentiation in a process that is efficiently blocked by IL-18 under experimental infection conditions. Prior vaccination overrides the regulatory effects of IL-18 and promotes protective immunity, leading to clearance or at least a significant reduction of bacterial loads. Both Th subsets are required for protective immunity (31) and probably for infection-associated immunopathology.
the notion that inflammasome activation mediates protection from intestinal inflammation. For instance, gut inflammation induced by S. typhimurium infection depends on the type III secretion system-dependent activation of the NLRC4 inflammasome in stromal cells (12). Older publications reported a crucial contribution of inflammasome/caspase-1 activation and IL-18 production by hematopoietic cells to both DSS-induced and 2,4,6-trinitrobenzene-sulfonic acid-induced colitis (42, 43). Therefore, we propose that gastrointestinal inflammation induced by microbial pathogens or the disruption of epithelial barrier function is influenced greatly by at least four independent parameters: 1) the (predominant) NLRs sensing the PAMPs or danger signals, 2) the cell type in which inflammasome activation occurs, 3) the target cell type of the caspase-1 substrates, and 4) the relative availability of pro–IL-1β and pro–IL-18 for cleavage by caspase-1. In Helicobacter infection and gastritis models, active IL-18 balances the proinflammatory activities of simultaneously produced mature IL-1β and, thereby, effectively prevents excessive Helicobacter-specific pathogenic Th17 responses and gastric preneoplastic immunopathology. Thus, our results provide experimental evidence for a predominantly regulatory, as opposed to proinflammatory, function of IL-18 in the gut and implicated Th17 cells as main targets of IL-18-mediated immune regulation.

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Disclosures

The authors have no financial conflicts of interest.

References


Supplemental Figures

Supplemental Figure 1

**Suppl. Fig. 1:** Caspase-1 is activated and IL-1β and IL-18 are secreted upon infection of BMDCs with *H. felis*, *H. pylori SS1* and *H. pylori PMSS1*. BMDCs derived from wild type (wt) or caspase-1−/− (casp1−/−) bone marrow were pretreated for 3h with 5ng/ml *E. coli* LPS and infected for 16h with the indicated strains at a multiplicity of infection of 50. *(A)* Supernatants and cell lysates were processed for Western blotting using an activated caspase-1-specific antibody. The pro-caspase-1 is visible in lysates of infected and uninfected wild type, but not caspase-1−/− DCs; the p10 auto-catalytically activated subunit of caspase-1 (marked with an asterisk) is detected only in the supernatants of infected wild type cells. *(B,C)* Supernatants of the infection experiment shown in *A* were subjected to IL-1β *(B)* and IL-18 *(C)* ELISA. Both cytokines are released by infected wild type, but not caspase-1−/− DCs.
Suppl. Fig. 2: Lack of caspase-1 improves the spontaneous control of Helicobacter infection, and aggravates gastric immunopathology. (A) Histopathology scores of wild type and caspase-1−/− mice infected with *H. felis* for 3 months; horizontal lines indicate the means. (B) *H. felis* colonization as determined by quantitative PCR of the *flaB* gene; medians are represented by horizontal lines.