The Role of Decay-accelerating Factor as a Receptor for Helicobacter pylori and a Mediator of Gastric Inflammation**

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Persistent gastritis induced by Helicobacter pylori is the strongest known risk factor for peptic ulcer disease and distal gastric adenocarcinoma, a process for which adherence of H. pylori to gastric epithelial cells is critical. Decay-accelerating factor (DAF), a protein that protects epithelial cells from complement-mediated lysis, also functions as a receptor for several microbial pathogens. In this study, we investigated whether H. pylori utilizes DAF as a receptor and the role of DAF within H. pylori-infected gastric mucosa. In vitro studies showed that H. pylori adhered avidly to Chinese hamster ovary cells expressing human DAF but not to vector controls. In H. pylori, disruption of the virulence factors vacA, cagA, and cagE did not alter adherence, but deletion of DAF complement control protein (CCP) domains 1–4 or the heavily O-glycosylated serine-threonine-rich COOH-terminal domain reduced binding. In cultured gastric epithelial cells, H. pylori induced transcriptional up-regulation of DAF, and genetic deficiency of DAF attenuated the development of inflammation among H. pylori-infected mice. These results indicate that DAF may regulate H. pylori-epithelial cell interactions relevant to pathogenesis.

Helicobacter pylori induces an inflammatory response in the stomach that persists for decades and biological costs incurred by chronic infection include an increased risk for peptic ulceration, gastric adenocarcinoma, and non-Hodgkins lymphoma of the stomach (1, 2). However, most colonized individuals remain asymptomatic, and increased disease risk is related to bacterial strain differences, epithelial responses governed by host diversity, and/or specific interactions between host and microbial determinants.

While the vast majority of H. pylori in colonized hosts are free-living, ~20% bind to gastric epithelial cells, and this adherence is required for induction of injury. The H. pylori cag pathogenicity island encodes a type IV secretion system that, following adherence, translocates peptidoglycan and CagA into host cells (3–8). CagA subsequently undergoes Src-dependent tyrosine phosphorylation and activates a eukaryotic phosphatase (SHP-2), eventually in dephosphorylation of host cell proteins and cellular morphological changes (6, 7, 9, 10). Recently, CagA has also been shown to activate β-catenin and induce NF-κB-mediated interleukin-8 release from gastric epithelial cells (11, 12). The presence of the cag island influences the topography of colonization, as H. pylori cag+ strains predominate within the mucus gel layer, while cag− strains are found immediately adjacent to epithelial cells (13). Concordant with these properties, H. pylori strains that harbor a functional cag island are associated with an increased risk for ulcer disease and gastric cancer compared with cag− strains (1).

Another H. pylori locus linked with pathologic outcomes is vacA, which encodes a bacterial toxin (VacA) that induces vacuolation and apoptosis of epithelial cells (14–16). VacA binds to a unique receptor-type protein-tyrosine phosphatase, PTPζ, a member of a family of receptor-like enzymes that regulate cellular proliferation, differentiation, and adhesion (17). Another virulence factor, an adhesin termed BabA (encoded by the H. pylori strain-selective gene babA2), binds the blood group antigen Lewisb on gastric epithelial cell membranes (18, 52), and H. pylori babA2+ strains increase the risk for gastric adenocarcinoma (19). Finally, genetic ablation of parietal cells in mice induces gastric epithelial progenitor cells to synthesize NeuAc2,3Galβ1,4 glycans, which serves as a receptor for H. pylori, and this is accompanied by an expansion of bacterial colonization and inflammation within the glandular epithelium (20, 21, 53). Collectively, these results indicate that dynamic and specific interactions between H. pylori and host receptors legislate pathologic outcome.

Decay-accelerating factor (DAF) is an intrinsic regulator of complement, which is attached to the outer leaflet of the cell membrane (22). It is a 70-kDa glycoprotein containing 4 contiguous 60-amino acid-long repeats termed complement control protein repeats (CCPs) followed by a serine-threonine-rich heavily O-glycosylated COOH-terminal domain that elevates the molecule at the membrane surface (22). DAF is membrane-linked by a glycosylphosphatidylinositol anchor. DAF protects self cells from complement activation on their surfaces by dissociating membrane-bound C3 convertases that are required for cleaving C3 and initiating further propagation of the complement cascade. Previous work has shown that DAF is utilized as a cellular receptor for several pathogenic organisms including uropathogenic diffusely adher-
ing *Escherichia coli*, coxsackieviruses, echoviruses, and enteroviruses (22–32). Studies by other investigators have shown that expression of DAF is increased within *H. pylori*-infected human gastric tissue compared with uninfected mucosa, and this increase is directly related to the density of *H. pylori* colonization and severity of inflammation (33–35). It has also been shown that increased DAF expression is present in gastric cancer precursor lesions such as intestinal metaplasia and gastric adenomas, and in gastric adenocarcinoma specimens compared with non-transformed gastric mucosa (36), suggesting that aberrant expression of DAF precedes the development of gastric cancer. Since DAF is overexpressed within *H. pylori*-associated premalignant and malignant lesions, we investigated whether *H. pylori* utilizes DAF as a receptor in *vitro* and the role of DAF within *H. pylori*-infected gastric mucosa to define a potential pathogenic response toward this organism.

**MATERIALS AND METHODS**

Recombinant Cell Lines—Chinese hamster ovary (CHO) cell transfectant clones that stably express human DAF cDNA (DAF/A9), cDNA CCP deletion constructs (DAFΔCCP1/029–6B, DAFΔCCP2/043–7A, DAFΔCCP3/044–2D, DAFΔCCP4/054–5 × 4), a cDNA serine/threonine (S/T)-rich region deletion construct (DAFΔS/T), a cDNA S/T region deletion construct containing an in-frame fusion of the cDNA encoding the amino-terminal region of DAF with the carboxyl terminus of HLA-B44 (DAFΔS/T + HLA), or vector alone were used as described previously (37). Cells were cultured at 37 °C in Ham’s nutrient mixture F-12 (Invitrogen) supplemented with 10% heat-inactivated fetal bovine serum (Sigma), 50 units of penicillin/ml, 50 μg of streptomycin/ml, 2 mM 1-glutamine, nonessential amino acids, and 250 μg of G418/ml when indicated. AGS (ATCC CRL 1739) or MKN28 (kindly provided by Dr. Robert Coffey, Vanderbilt University) human gastric epithelial cells were grown in RPMI 1640 (Invitrogen) with 10% heat-inactivated fetal bovine serum and 20 μg/ml gentamicin in an atmosphere of 5% CO₂ at 37 °C.

Bacterial Strains Used in Vitro—Experiments were performed with the cag + toxigenic *H. pylori* strain J166, as well as eight additional (5 cag + toxigenic, 3 cag - non-toxigenic) well characterized clinical strains. Clinical strains were selected from a larger population of isolates that have been previously described as part of an ongoing prospective study designed to study mechanisms of *H. pylori* pathogenesis (15). Since we sought to analyze the importance of *H. pylori* genes related to disease, we selected strains that varied in cag status and toxin production. Iso- genic cagA, cagE, and vacA null mutants were constructed within strain J166 by insertional mutagenesis, using aphA (conferring kanamycin resistance) as described previously (15, 38), and were selected on Brucella agar with kanamycin (25 μg/ml). As bacterial controls, *Campylobacter jejuni* strain 81176 and a non-diffusely adhering flagellated *E. coli* strain (HB101) were also co-cultured with CHO cells (38).

Recombinant CHO cells expressing full-length DAF, its domain deletion mutants, or vector alone were seeded in 100-mm polypropylene tissue culture dishes (Nunc) at 2.5 × 10⁵ cells/dish and allowed to grow for 24 h to subconfluence. *H. pylori* were grown in Brucella broth with 10% FCS for 18 h, harvested by centrifugation, resuspended to a concentration of 1 × 10⁹ colony forming units (cfu)/ml, and added to cells at a bacteria:cell ratio of 10:1 (39). Co-culture experiments with viable *H. pylori* were performed in antibiotic-free medium with 10% heat-inactivated fetal bovine serum. For quantitative culture of adherent bacteria, *H. pylori*:CHO cell co-cultures were washed after 4 h with phosphate-buffered saline (PBS; pH 7.6) × 2 to remove non-adherent bacteria, and total cell extracts were harvested as described (38). Serial 10-fold dilutions of 1-ml aliquots of cell extracts were cultured on 5% sheep blood agar plates and incubated for 3–5 days under microaerobic conditions before *H. pylori* colonies were counted. Results are expressed as cfu/ml.

**Immunofluorescence**—CHO or gastric cells were cultured on glass cover slides, and cells treated with or without *H. pylori* for 4 h (multiplicity of infection = 100) were washed twice with PBS, fixed in 4% paraformaldehyde in PBS for 20 min at room temperature, incubated in 0.1% PBS/T (1 × PBS; pH 7.6, 0.1% Tween 20) with 5% milk for 1 h, and then incubated with mouse monoclonal anti-DAF antibody 1H4 (1:100) (37) for 1 h. For dual immunofluorescence, slides were stained with mouse monoclonal anti-DAF antibody 1H4 (1:100) and rabbit anti-*H. pylori* antibody (1:100, DakoCytomation). Washed slides were then incubated with either goat anti-mouse Alexa Fluor 546-conjugated antibody (1:100; Molecular Probes) for single immunofluorescence or Alexa Fluor 488-conjugated goat anti-rabbit antibody and Alexa Fluor 546-conjugated goat anti-mouse antibody (1:100, Molecular Probes) for dual immunofluorescence at room temperature for 1 h. Nuclei were stained using 4’,6-diamidino-2-phenylindole. Slides were mounted using Vectashield mounting medium (Vector Laboratories, Burlingame, CA), and immunofluorescence was observed using a fluorescence microscope (40). For antibody inhibition studies, CHO cells were preincubated with anti-DAF monoclonal antibodies 11D7 (directed against CCP domain 1), 1H4 (directed against CCP domain 3), 8D11 (directed against CCP domain 4) (37), or ascites fluid containing the monoclonal antibody IF7 (directed against CCP domain 2) (25) for 30 min prior to infection with *H. pylori*.

**Western Analysis**—Transfected CHO cells or gastric cells from *H. pylori*:AGS or *H. pylori*:MKN28 cell co-cultures were lysed in RIPA buffer (50 mM Tris, pH 7.2, 150 mM NaCl, 1% Triton X-100, 0.1% SDS), and protein concentrations were quantified by the Bradford assay (Pierce) (38). Proteins (20 μg) were separated by SDS-PAGE and transferred to polyvinylidene difluoride membranes (Pall Corp., Ann Arbor, MI). DAF levels were measured in gastric cells by Western blotting using anti-DAF (1:1000, 1H4) antibodies (38). Primary antibodies were detected using goat anti-mouse (Santa Cruz Biotechnology) horseradish peroxidase-conjugated secondary antibodies and visualized by Western Lightning Chemiluminescence Reagent Plus (PerkinElmer Life Sciences) according to the manufacturer’s instructions.

**Real-time PCR**—MKN28 and AGS gastric epithelial cells were grown to confluence, serum-starved for 24 h, and then co-cultured with *H. pylori* for 2, 6, 12, and 24 h (multiplicity of infection = 100). RNA was prepared from *H. pylori*:gastric cell co-cultures using TRIzol Reagent following the manufacturer’s instructions (Invitrogen), and contaminating DNA was removed using the RNaseasy RNA purification kit (Qiagen). Reverse transcriptase PCR was performed using TaqMan reverse transcription reagents (Applied Biosystems), which was followed by real-time quantitative PCR using the TaqMan gene expression assay and a 7300 real-time PCR system (Applied Biosystems). DAF cDNA was quantitated using a DAF TaqMan gene expression primer set purchased from Applied Biosystems and expression levels were normalized to levels of 18 S rRNA.

**Mice, Bacteria, and Experimental Infections**—Daf1 knock-out mice were developed as described previously (41). Briefly, the Daf1 gene on one chromosome was inactivated by homologous recombination and Cre/LoxP-mediated deletion in murine GK129 embryonic stem cells. The recombined embryonic stem cells were microinjected into blastocysts, chimeras were generated, and the chimeric mice were then bred with the C57BL/6 strain. Eight- to 12-week knock-out mice and wild-type C57BL/6 mice were used. All experiments were approved by the Case Western Reserve Institutional Animal Care and Use Committee.

Brucella broth containing 2 × 10⁷ cfu of the *H. pylori* rodent-adapted
strain SS1 was used as inoculum and was delivered by gastric intubation as described previously (42).

Eight weeks post-challenge, mice were euthanized. At necropsy, linear strips extending from the squamocolumnar junction through proximal duodenum were fixed in 10% neutral buffered formalin, paraffin-embedded, cut at 5 μm, and stained with hematoxylin and eosin. Indices of inflammation and injury in the gastric cardia, corpus, and antrum were scored on an ordinal scale from 0 to 5 in increments of 0.5 by a single veterinary pathologist blinded to treatment groups as described previously (42).

For quantitative culture, gastric tissue was homogenized, plated, and incubated under microaerobic conditions at 37 °C for 5–6 days as described previously (42). After verification by Gram's stain, urease, catalase, and oxidase reactions, colonies were counted and comparisons between groups were based on the log cfu/gram of stomach tissue as described (42).

Statistical Analysis—The Mann-Whitney U test was used for statistical analyses of intergroup comparisons. Significance was defined as \( p \leq 0.05 \).

RESULTS

Expression of Human DAF Increases Cellular Binding of \( H. pylori \) in Vitro—To determine whether DAF mediates \( H. pylori \) binding to host cells, we used CHO cells stably transfected with a human DAF cDNA or vector alone (Fig. 1A) and co-cultured the transfectants with a well characterized \( H. pylori \) strain, J166, which is easily transformable and binds well to gastric epithelial cells (15, 43). We assessed binding by quantitative culture. Compared with cells lacking DAF, \( H. pylori \) strain J166 bound more avidly to DAF-expressing cells and recoverable colony-forming units were \( \geq 1 \) log-fold higher following only 4 h of coculture (Fig. 1B).
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To further assess DAF binding by H. pylori, we used immunofluorescence. For this, strain J166 was co-cultured with CHO cells that either did or did not express DAF. A significantly greater number of H. pylori adhered to DAF+ versus DAF− CHO cells (Fig. 1C).

To ensure that H. pylori binding to DAF was a specific interaction rather than a nonspecific bacterial response of the cells to bacteria, we incubated DAF+ and DAF− CHO cells with equivalent numbers of non-diffusely adhering E. coli or C. jejuni for 4 h and measured binding by quantitative culture. Adherence of E. coli or C. jejuni did not differ between CHO cells that either expressed or did not express DAF (Fig. 1B). Thus, the DAF interaction specifically mediated adherence of H. pylori to host cells.

Binding to DAF Is Independent of H. pylori Virulence Constituents Encoded by the cag Pathogenicity Island or vacA—The H. pylori cag island and vacA induce epithelial responses that may lower the threshold for disease. Consequently, we examined the effects of these virulence determinants on binding of H. pylori to DAF-expressing cells. To do this, we co-cultured DAF+ or DAF− CHO cells with H. pylori wild-type cag+ toxicogenic strain J166 or isogenic cagA, cagE, or vacA null mutant derivatives. Compared with the parental wild-type strain, loss of cagA had no effect on binding to DAF (Fig. 2A). Inactivation of cagE or vacA decreased the extent of bacterial binding compared with the wild-type strain; however, the level of reduction was similar in DAF-expressing and DAF-deficient CHO cells (Fig. 2A). These results indicate that, although cagE and vacA may contribute to binding of H. pylori to host cells, these effects do not involve DAF.

The Extent of H. pylori Binding to DAF-expressing Cells Varies among a Population of Clinical Isolates—Most persons infected with H. pylori cag+ toxicogenic strains remain asymptomatic, suggesting that additional microbial and/or host factors influence disease. Therefore, we next investigated DAF binding patterns among a population of clinical H. pylori isolates. Although absolute levels varied between different isolates, all nine strains tested (6 cag+ toxicogenic, 3 cag− non-toxicogenic) displayed at least a 10-fold increase in binding affinity to DAF+ versus DAF− CHO cells (Fig. 2B). Levels of binding did not segregate with cag genotype or toxigenicity, confirming the results from isogenic mutant experiments (Fig. 2A).

 Binding of H. pylori to CHO Cells That Express Mutant DAF—DAF is composed of four CCPs, and microbial pathogens vary in their utilization of CCP domains for binding. Based on this, we next localized the sites on DAF that H. pylori utilize by studying CHO cells stably transfected with deletion mutants of each of the four DAF CCP domains. Each deletion mutant expresses an amount of DAF at least equal to cells expressing wild-type human DAF (wild-type (WT)), a series of deletion mutants that individually lack one of the four DAF CCP domains, a S/T-rich region deletion mutant (ΔS/T), a ΔS/T-rich region deletion mutant containing an in-frame fusion of the cDNA encoding the amino-terminal region of DAF with the carboxyl terminus of HLA-B44 (ΔS/T + HLA), or vector alone. Bacterial adherence was assessed using quantitative culture as described under “Materials and Methods” and is expressed as a ratio of H. pylori recovered from DAF+ versus DAF− cells. Therefore, a value of 1 represents baseline. Error bars, S.E.; *, p < 0.005 versus infected DAF− cells. Binding of H. pylori to CHO cells transfected with either vector alone (DAF−) or full-length human DAF (DAF+) in the presence or absence of an equal mixture of anti-DAF CCP-specific monoclonal antibodies 11D7, IF7, 1H4, or 8D11, or an irrelevant anti-Myc monoclonal antibody was detected by immunofluorescence as described under “Materials and Methods.” Error bars, S.E.; *, p < 0.005 versus infected DAF− cells.

FIGURE 2. Adherence of H. pylori to DAF-expressing cells is not mediated by the cag pathogenicity island or vacA. A, CHO cells transfected with either full-length DAF (DAF+), or vector alone (DAF−) were cultured in the presence of the wild-type (wt) H. pylori cag+ toxicogenic strain J166 or isogenic cagA, cagE, or vacA null mutant derivatives at bacteria:cell ratios of 10:1. Adherence was assessed by quantitative culture. Error bars, S.E.; *, p < 0.05 versus infected DAF− cells. B, adherence to DAF+ or DAF− CHO cells by H. pylori clinical isolates with varying cag island status and toxigenic phenotypes was assessed using quantitative culture. Results are expressed as a ratio of H. pylori recovered from DAF+ versus DAF− cells. A representative result of multiple repetitions performed on at least two occasions is shown.

FIGURE 3. Adherence of H. pylori to DAF-expressing cells is mediated by multiple DAF domains. A, H. pylori strain J166 was co-cultured with CHO cells stably transfected with either full-length human DAF (wild-type (WT)), a series of deletion mutants that individually lack one of the four DAF CCP domains, a S/T-rich region deletion mutant (ΔS/T), a ΔS/T-rich region deletion mutant containing an in-frame fusion of the cDNA encoding the amino-terminal region of DAF with the carboxyl terminus of HLA-B44 (ΔS/T + HLA), or vector alone. Bacterial adherence was assessed using quantitative culture as described under “Materials and Methods” and is expressed as a ratio of H. pylori recovered from DAF+ versus DAF− cells. Therefore, a value of 1 represents baseline. Error bars, S.E.; *, p < 0.05 versus infected DAF− cells.
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To further confirm the role of CCP domains for *H. pylori* binding, DAF<sup>+</sup> and DAF<sup>−</sup> CHO cells were pretreated with the DAF CCP-specific monoclonal antibodies 11D7, 1F7, I4H, and 8D11 prior to infection with *H. pylori* strain J166. Preincubation with each individual monoclonal antibody alone did not significantly reduce *H. pylori* binding to DAF<sup>+</sup> cells (data not shown). However, preincubation with an equal mixture of the four CCP domain-specific monoclonal antibodies completely blocked adherence of *H. pylori* to DAF-expressing cells (Fig. 3B), confirming the results using cells transfected with DAF CCP deletion mutants (Fig. 3A).

DAF CCP domains are linked to a S/T-rich heavily O-glycosylated COOH-terminal domain that elevates the molecule at the membrane surface. Since *H. pylori* can bind to carbohydrate residues on cell surfaces (1, 2), we determined the requirement for the DAF S/T region by utilizing a CHO cell transfectant clone that stably expresses a DAF cDNA construct containing a deletion of the S/T-rich domain or a clone expressing a S/T deletion construct that attaches the four CCP domains to the unrelated non-complement protein HLA-B44. The latter construct is anchored by the transmembrane and cytoplasmic domains of HLA-B44 and functions efficiently as a complement regulatory protein. Results from binding experiments using both of these S/T-deficient DAF clones demonstrate that removal of the O-glycosylated region decreased *H. pylori* binding to DAF (Fig. 3A), indicating that the S/T region does not simply function as a nonspecific spacer for binding. These results indicate that *H. pylori* binding to DAF either involves all CCP domains or is dependent on the conformation of DAF in its intact state.

*H. pylori* Induces DAF Expression in Human Gastric Epithelial Cells—Since *H. pylori* is a human pathogen that selectively colonizes gastric epithelium, we investigated whether *H. pylori* alters DAF expression in human gastric epithelial cells. Real-time PCR analysis demonstrated that *H. pylori* induced DAF expression in MKN28 (Fig. 4A) and AGS (data not shown) gastric epithelial cells beginning at 2 h. Levels decreased to base line by 24 h post-inoculation. *H. pylori* co-culture mRNA changes reflected increased DAF protein expression, since increases in levels were detected at 6 h and DAF protein remained elevated for 24 h (Fig. 4B). Immunofluorescence staining confirmed the increased DAF expression on MKN28 gastric epithelial cells following co-culture with *H. pylori* (Fig. 4C). These results indicate that the prototypic *H. pylori* strain J166 induces transcriptional up-regulation of DAF in human gastric epithelial cells.

*H. pylori*-induced Gastric Inflammation Is Attenuated in the Absence of DAF—To determine whether the DAF binding is physiologically relevant within the context of *H. pylori*-induced inflammation in vivo, we utilized Daf<sup>−/−</sup> mice and the rodent-adapted *H. pylori* strain SS1. We infected wild-type and Daf<sup>−/−</sup> mice in two independent challenges and followed disease outcome. All mice challenged with *H. pylori* were successfully infected and there were no differences in colonization efficiency or density between wild-type and DAF deficient mice (Fig. 5A).

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**FIGURE 4. H. pylori strain J166 up-regulates DAF in gastric epithelial cells.** A, MKN28 gastric epithelial cells were cultured for 24 h prior to incubation with *H. pylori* strain J166. Levels of DAF mRNA were determined by real-time RT-PCR as described under “Materials and Methods” and were normalized to corresponding levels of 18 S rRNA. Results are expressed as fold increase in DAF mRNA in *H. pylori*-infected versus uninfected samples. Error bars, S.E.; , p < 0.05 versus uninfected cells at corresponding time point. B, MKN28 cells were cultured for 24 h prior to incubation with *H. pylori* strain J166. Cell extracts harvested at different time points were then used for Western blot analysis using an anti-DAF antibody as described under “Materials and Methods.” , cells incubated with medium alone. A representative blot of multiple repetitions performed on at least three occasions is shown. Anti-actin blots served as normalization controls for MKN28 cell viability under different experimental conditions. C, distribution of DAF (red) in MKN28 gastric epithelial cells was detected by immunofluorescence following infection with medium alone (left panel) or *H. pylori* strain J166 (right panel) for 24 h.
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Eight weeks post-challenge, there were few lesions in the stomachs of wild-type or Daf1−/− mice inoculated with broth alone (Fig. 5B), whereas, as expected, wild-type mice challenged with H. pylori developed gastritis. Inflammation was most extensive at the transition zones between the antrum or cardia and the corpus. Inflammatory cells within infected mucosa consisted of polymorphonuclear cells and large mononuclear cells in the lamina propria (Fig. 6). In the lamina propria, the infiltrate separated and displaced the glands (Fig. 6). Gastric pits were lengthened and were lined by less mature flattened epithelial cells with basophilic cytoplasm; mitotic figures were frequently identified. In the submucosa, edema often accompanied the cellular infiltrate (Fig. 6).

In contrast to infected wild-type mice, in H. pylori-colonized DAF-deficient mice, the intensity of inflammation was significantly attenuated (p = 0.013) (Figs. 5B and 6). Moreover, there were no differences in severity of gastritis between infected and infected Daf1−/− mice (p = 0.53; Fig. 5B). These results thus indicate that DAF contributes to the ability of H. pylori to induce injury within the gastric niche.

DISCUSSION

Colonization of humans by pathogenic bacteria is common, but disease occurs in only a fraction of infected persons. Our current experiments identify a new mechanism that may contribute to H. pylori pathogenesis. This insight was gained by 1) capitalizing on a recombinant cell model to demonstrate that the protein DAF serves as a receptor for H. pylori, 2) finding that H. pylori can induce DAF expression in a biologically relevant in vitro model of microbial/gastric epithelial cell interaction, 3) both confirming and mapping the components of DAF required for these effects, and 4) through the use of a Daf1−/− knock-out mouse, documenting that the interaction is important for pathogenesis. Collectively, these studies indicate that H. pylori co-opts DAF as a receptor to induce disease.

The hallmark of the gastric inflammatory response to H. pylori is its capacity to persist for decades. This is in contrast to inflammatory reactions induced by other mucosal pathogens, such as Salmonella, that either resolve within days or progress to eliminate the host. Research to date has shown that H. pylori has evolved numerous strategies to facilitate its persistence within the stomach including limiting the bacterial effects of pro-inflammatory molecules (44) and varying the antigenic repertoire of surface-exposed proteins (45). Adherence of H. pylori to gastric epithelial cells is also critical for colonization. According to our data, the ability of H. pylori to utilize DAF as a receptor contributes to the latter strategy and is consistent with the role that this molecule plays in other host-microbial interactions involving persistent pathogens. E. coli that express DAF-binding Dr adhesins cause chronic intestinal nephritis (46). Echoviruses and coxsackieviruses that target DAF as a receptor are associated with chronic fatigue syndrome and chronic dilated cardiomyopathy, respectively (47, 48). The current studies focused on H. pylori-DAF interactions further implicate DAF as a receptor that is exploited by pathogens notable for their ability to induce chronic inflammation, injury, and disease.

Our in vitro results indicate that binding of DAF by H. pylori requires all of the CCP domains and the S/T-rich O-glycosylated region, a pattern that is distinct from those involved in DAF binding by other pathogens. For example, Dr-expressing E. coli require CCP2 and CCP3 (23–25). E. coli that express X adhesins require CCP3 and CCP4 (26). Echovirus 7 utilizes CCPs 2–4 (27, 28), and coxsackieviruses A21 and B3 exploit CCP1 and CCP2, respectively (29–31). Another layer of complexity beyond the scope of this investigation is added when results from inhibition studies are considered. Anti-DAF antibodies that block cellular binding of coxsackievirus 21 reciprocally enhance binding of echovirus 7 (32), raising the possibility that ligand binding of one CCP domain affects binding of another CCP moiety within the same DAF molecule. Importantly, we have a unique in vitro model of bacteria epithelial interactions using an H. pylori strain that is easily transformable in which to evaluate the individual and collective effects of each of these factors.

Murine models have provided valuable insights into the host, bacterial, and environmental factors involved in H. pylori-induced gastric inflammation and injury. Using a Daf1−/− mouse, we now demonstrate that loss of DAF does not alter colonization but attenuates the inflammatory response to H. pylori. This may occur via more than one mechanism. Our available data so far indicate that H. pylori up-regulates DAF in gastric epithelial cells. This is consistent with reports from other
investigators that DAF expression is increased within infected human gastric mucosa, where it localizes to the apical surface of gastric epithelial cells (33–35). One possibility raised by our findings is that in addition to direct bacterial stimulation, expression of DAF can be increased by *H. pylori*-induced pro-inflammatory cytokines such as interleukin-1β and tumor necrosis factor-α, which are up-regulated in response to transmperial migration of neutrophils (49, 50). DAF has also been recently identified as an apical epithelial ligand for polymorphonuclear cells that regulates the rate of neutrophil migration across apical epithelial membranes (51). Finally, since *H. pylori* binding to DAF involves its complement regulatory CCP2 and CCP3 domains, the binding might affect complement activation. All these questions will require further study. Thus, DAF regulated by both *H. pylori* and host immune mediators is well positioned to modulate the inflammatory response to this pathogen.

In conclusion, *H. pylori* binds avidly to cells expressing human DAF and this is mediated by DAF CCP1–4 and the O-glycosylated serine/threonine-rich COOH-terminal domain. *H. pylori* induces transcriptional up-regulation of DAF in gastric epithelial cells, and *in vivo* DAF deficiency decreases the intensity of inflammation in *H. pylori*-infected mice. Taken together, these data open a new avenue of investigation in pathogenic mechanisms underlying *H. pylori* infection.

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