The complement regulator CD46 is bactericidal to *Helicobacter pylori* and blocks urease activity

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ABSTRACT

Helicobacter pylori colonizes the gastric mucosa and infection may lead to gastritis, peptic ulcer disease and adenocarcinoma. Here we report that *H. pylori* infection causes shedding of the human complement regulator CD46. Further, we show that a soluble form of CD46 binds to *H. pylori* and inhibits growth in a dose- and time-dependent manner by interacting with urease and alkyl hydroperoxide reductase (AhpC), two essential bacterial virulence factors. Binding of CD46 or CD46-derived synthetic peptides blocks urease activity of bacteria, and ability to survive in acidic environments. In addition, oral administration of one CD46-peptide eradicates *H. pylori* from infected mice. The results unveil a new paradigm whereby CD46 can eradicate *H. pylori* as an antimicrobial agent. Taken together, these data reveal a novel bactericidal role of CD46 and suggest CD46-peptides as a new therapeutic strategy for treating *H. pylori* infection.

INTRODUCTION

Helicobacter pylori is a gram-negative bacterium that colonizes the human gastric and duodenal mucosa of more than half of the world's population. The bacteria are found associated with the mucous layer as well as attached to the gastric epithelium[1,2]. *H. pylori* infection is often acquired during childhood and is spread through person-to-person contact or by ingesting contaminated food and water. The bacteria persist in the gastric environment for decades and even throughout the life span of the host[3]. *H. pylori* infection is the strongest known risk factor for gastroduodenal ulcers, and is the first bacterium defined as a causative agent of gastric adenocarcinoma and gastric mucosa-associated lymphoid tissue lymphoma[4].

H. pylori possesses a number of different colonization and virulence determinants. The urease enzyme, encoded by *ureA* and *ureB,* neutralizes gastric acidity by degrading urea at the site of colonisation[5], and the bacterial flagella mediate motility[6]. The *VacA* cytotoxin[7,8] and *CagA* pathogenicity island contribute to cytotoxicity and affect epithelial cell viability[9,10]. The best-studied *H. pylori* adhesins are outer membrane proteins that bind carbohydrate structures on host cell glycoproteins. The *BabA* adhesin binds fucosylated blood group antigen Lewis-b[11] and the sialic acid-binding adhesin SabA, binds to sialylated carbohydrate structures on gastric epithelial cells[12]. Several other outer membrane proteins, such as HopZ, HopH, AlpA and AlpB, have also been shown to mediate bacterial attachment to host cells[13,14].

The complement system of the host has a crucial role in eliminating pathogenic organisms. Many bacteria have developed the ability to escape the machinery of the human complement system by interfering with complement regulatory factors. CD46, a human cell surface glycoprotein expressed by virtually all cells except erythrocytes,

regulates complement activation by serving as a cofactor in the proteolysis of deposited C3b and C4b by serine protease factor I. CD46 consists of four homologous complement control protein repeats (CCPR-1, -2, -3, and -4), a serine-threonine-proline (STP)-rich domain, a transmembrane hydrophobic domain, a cytoplasmic anchor and a cytoplasmic tail. Four major isoforms of CD46 are expressed due to alternative splicing and the choice between one of two cytoplasmic tails, Cyt-1 and Cyt-2[15-17]. CD46 interacts with adenovirus[18], human herpes virus 6[19], pathogenic *Neisseria*[20-22], measles virus[23] and *Streptococcus pyogenes.*[24] Infection of epithelial cells by *N. gonorrhoeae* and *S. pyogenes* triggers shedding of CD46[25,26]. Although CD46 is expressed in human gastric epithelium[27], the role of CD46 in *H. pylori* infection has not yet been elucidated.

In this study, we show that *H. pylori* triggers shedding of CD46 from host cells. Also, we provide evidence that a soluble form of CD46 binds to *H. pylori* and inhibits growth in a dose and time dependent manner. The bactericidal activity was retained in a 24 amino acid synthetic peptide derived from CD46, which also inhibited the urease activity. Finally, we demonstrate that oral administration of the CD46-peptide eliminates *H. pylori* infection in a mouse model of disease. Taken together, this study demonstrates a novel function of CD46 as a virulence blocker that interferes with the capacity of the pathogen to survive in the acidic gastric mucosa.

RESULTS

H. pylori induces shedding of CD46 from human gastric epithelial cells

To determine if infection by *H. pylori* resulted in modulation of CD46 expression, we used AGS gastric epithelial cells, which express high levels of CD46 (**Supplementary Fig. 1a**), and three *H. pylori* wild-type strains, J99, HPAG1 and 26695. At 18 h post infection, all three strains adhered to AGS cells (**Fig. 1a**). Analysis by flow cytometry revealed that *H. pylori* infection dramatically reduced the CD46 amount at the cell surface (**Fig. 1b).** To assess whether the loss of CD46 was due to internalization or shedding of the protein, the supernatants of infected and uninfected AGS cells were analyzed in ELISA. The amount of CD46 was more than 6-fold increased in supernatants of infected AGS cells compared to uninfected cells (**Fig. 1c**). These data argue that *H. pylori* infection promotes shedding of CD46 from human gastric epithelial AGS cells. Elwards *et al*. 2005[28] showed that chemical stimulation of apoptosis and necrosis results in shedding of CD46 from various human cell lines, whereas other cell surface markers remain stable at the cell surface. It is known that *H. pylori* induces apoptosis in human gastric epithelial cells [7,8,29-31]. Indeed, staining with propidium iodine and analysis by flow cytometry revealed that 60% of cells were positive at 18 h post infection (**Supplementary Fig. 1b**), suggesting that shedding of CD46 is linked to apoptosis.

H. pylori binds to recombinant CD46 (CD46)

Since CD46 is released from gastric epithelial cells after *H. pylori* infection, we hypothesized that *H. pylori* might bind CD46. To investigate whether CD46 interacts with *H. pylori*, we measured binding of purified CD46 to bacteria. Recombinant CD46, composed of the extracellular domain of the protein (**Fig. 2a**), was generated

as a thioredoxin-CD46 fusion protein (**Supplementary Fig. 2a**). Binding of CD46 to *H. pylori* was first assessed by a microtiter plate assay in which bacterial lysates were overlaid with CD46 followed by anti-CD46 antibodies. All three tested *H. pylori* strains tested bound CD46, however, strain J99 and HPGA1 bound better than strain 26695 (**Fig. 2b**). We next analyzed binding of CD46 to bacteria by flow cytometry and confirmed that all three *H. pylori* strains bind to CD46 even though the binding-level varies slightly between the strains. As negative control we used nonpathogenic *E. coli,* which failed to bind CD46, indicating specificity for *H. pylori* (**Fig. 2c**). In addition, we could not detect interaction between CD46 and *Pseudomonas aeruginosa* or *Campylobacter jejuni* (**Supplementary Fig. 2b**).To further study the interaction between CD46 and *H. pylori*, we designed a competition assay using the CD46-binding ligand C3b. Strain J99 was selected for these studies since it demonstrated slightly better binding to CD46 compared to the other strains (according to **Fig. 2c**). Recombinant CD46 was pre-incubated with C3b or anti-CD46 antibodies for 1 h, and then added to bacteria. Flow cytometry analysis showed that both C3b and CD46 antibodies blocked binding of CD46 to *H. pylori* (**Fig. 2d**). These data suggest that C3b and *H. pylori* recognize overlapping or closely linked CD46-domains. We next wanted to directly visualize the interaction between CD46 and bacteria by microscopic examination. Analysis by immunofluorescence microscopy confirmed binding of CD46 to *H. pylori* (**Fig. 2e**), but revealed an unexpected alteration of bacterial morphology, including loss of the bacterial spiral shape, increased bacterial aggregation and bacterial lysis upon incubation with CD46 (**Fig. 2f)**. The control Trx protein did not promote morphological changes of the bacteria. Taken together, these data demonstrate that *H. pylori* recognizes CD46 in a species-specific manner and indicate that CD46 is bactericidal against *H. pylori*.

CD46 inhibits growth of *H. pylori*

The bactericidal effect of CD46 was further evaluated by incubating *H. pylori* J99 with different concentrations of CD46 for 6 h. Addition of CD46 impaired bacterial survival in a dose dependent manner, whereas the control protein thioredoxin (Trx) had no bactericidal effect (**Fig. 3a**). We next investigated bacterial survival over time by incubating a fixed concentration of *H. pylori* with CD46 for 0 h, 6 h, and 24 h. Clearly, bacterial survival decreased by longer incubation time (**Fig. 3b**). Thus, CD46 inhibits growth of *H. pylori* in a dose and time dependent manner. To further study the bactericidal effect of CD46, *H. pylori* was preincubated with CD46 for 2 h, stained with propidium iodide (PI) and analyzed by flow cytometry. PI is normally not cell permeable and stains only DNA of non-viable bacteria. Bacteria pretreated with CD46 showed positive PI staining, suggesting permeabilization of membranes (**Fig. 3c**). PI-staining was not observed for bacteria incubated with thioredoxin, or with CD46 pre-incubated with C3b. These data demonstrate that CD46 binding to *H. pylori* leads to bacterial membrane leakage, and that C3b can block this bactericidal effect.

CD46 interacts with UreA and AhpC

In order to find bacterial proteins that bind to CD46, we passed whole bacterial extracts of strain J99 through Talon affinity columns pre-conjugated with recombinant CD46. The eluted fraction was separated by SDS-PAGE and stained with Coomassie brilliant blue (**Fig. 3d**). This revealed two apparent bands of 26 kD and 21 kD, which were identified as UreA and AhpC (alkyl hydroperoxide reductase) by mass spectrometry, respectively. The urease of *H. pylori* is composed of UreA and UreB and is an important virulence factor required for bacterial survival in the acidic gastric environment[32]. AhpC acts as a peroxide reductase in reducing organic

hydroperoxides and as a molecular chaperone for prevention of protein misfolding under oxidative stress[33,34]. To further confirm that UreA and AhpC are ligands of CD46, we generated a *ΔureA* mutant and a double *ΔureA/ΔahpC* mutant of strain J99. The strains were allowed to bind CD46 and then analyzed by flow cytometry. Deletion of both *ureA* and *ahpC* completely abolished binding of CD46 to bacteria, whereas deletion of *ureA* partially decreased CD46-binding, suggesting that both UreA and AhpC participate in the interaction with CD46 (**Fig. 3e**). To evaluate if binding of CD46 to UreA inhibits urease activity, total protein extracts of *H. pylori* J99 were first incubated with CD46 for 1 h, and then analyzed for urease activity. This resulted in more than 96% reduction of urease activity compared to the control (**Fig. 3f**). These data suggest that CD46 inhibits the urease activity by directly targeting UreA.

Identification of CD46 peptides with bactericidal effects towards *H. pylori*

To identify CD46 domain(s) with inhibiting activity against *H. pylori*, four peptides corresponding to hydrophilic, potentially exposed CCPR regions of CD46 were synthesized (**Fig. 4a)**. Bacteria were incubated with each peptide for 6 h and the bactericidal effect was evaluated. Peptide P1 and P2 had no detectable effect on bacterial survival, while both peptide P3 and P4 inhibited bacterial growth (**Fig. 4b**). The P3-peptide was the most efficient inhibitor by killing 80% of the bacteria. This made us focus further studies towards this peptide. Incubation of bacteria with peptide P3 for 6 h demonstrated a dose dependent bactericidal effect with 40% killing at 5 μM peptide and 80% at 30 μM peptide (**Fig. 4c**). These data suggest that the strongest interaction with *H. pylori* occurs within CCPR-3 region of CD46, which is also the previously reported binding region to C3b.

To verify that also the P3-peptide interacts with UreA and AhpC, FITC-labeled peptides were incubated with wild-type, *ΔureA*, and *ΔureA/ΔahpC* and analyzed by flow cytometry. The P3-peptide bound to *H. pylori* in a similar pattern as CD46, *i .e. ΔureA* bound to 47% as compared to wild-type and *ΔureA/ΔahpC* did not bind. The control peptide P1 and a random peptide (RP) did not bind to bacteria (**Fig. 4d**). We next analyzed urease activity in *H. pylori* J99 lysates after incubation with peptide P3 and peptide P1 (control). The P3-peptide, but not the P1 peptide, inhibited urease activity of J99 (**Fig. 4e**), suggesting that the P3-peptide, similar to CD46, inhibits an essential virulence property, *i. e.* urease activity, most likely by directly targeting UreA. Taken together, we have identified a 24 amino acid P3-peptide with ability to inhibit growth and urease activity of *H. pylori*.

Molecular docking CD46 to UreA and AhpC

To further study the interaction between CD46 and *H. pylori*, we performed molecular docking simulations of CD46 to urease and AhpC. Molecular docking of CD46 to urease suggests putative contact points at the assembly holes close to the active sites, primarily involving the P3 peptide domain (**Fig. 5a-d, Supplementary Table 1**). Similarly, this modelling suggests binding of CD46 to active assembly surfaces of AhpC, which could compete with the reductase function of AhpC (**Fig. 5e-g**). These predictions and modelling data support that binding of CD46, and especially peptide P3, occurs at sites that are likely to affect either active sites of the urease or the functional region of AhpC.

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Oral administration of P3-peptide eliminate *H. pylori* in a mouse model system

Since urease activity is essential for bacterial survival at the acidic gastric mucosa, we next evaluated whether the P3-peptide could affect *H. pylori in vivo* colonization of

mice. We used *H. pylori* strain 67:21 for the *in vivo* experiments, since this strain colonizes the gastric mucosa of mice, in contrast to J99. Strain 67:21 exhibited similar binding and interaction with CD46 and the P3-peptide as compared to J99 (**Supplementary Fig. 3**). The interaction between *H. pylori* and CD46 was not altered by changes in pH (**Supplementary Fig 2c**). To mimic a more human-like stomach, we used transgenic mice expressing CD46 in a human-like manner[35]. CD46 transgenic mice were infected orally with *H. pylori* once per day for 3 days. At 8 weeks post infection, colonization of *H. pylori* was detected by immunohistochemistry at the gastric mucosa. The mice were then orally fed with 20 μg P3-peptide suspended in water once per day for 2 weeks, whereas the control mice were fed with water in the same way. At the end of the peptide treatment, stomach tissue were collected, homogenized and plated for viable counts. Mice fed with water (-P3) showed high bacterial loads of 10^5 CFU/g, whereas bacteria were not detected in P3-treated mice (+P3), supporting that the P3-peptide had eradicated *H. pylori* from the mouse stomach (**Fig. 6a**). Further, tissue sections from stomach of P3-treated, untreated and uninfected (control) mice were examined for bacterial signals by fluorescence microscopy using *H. pylori* specific antibodies. *H. pylori* was detected at the gastric surface mucosa in non-treated mice; however, P3-treated and control mice showed no detectable *H. pylori* (**Fig. 6c**).

It has been reported that CD46 protein level at the gastric mucosa is decreased in humans infected with *H. pylori*[36]. We therefore stained the tissue sections with CD46 antibodies and revealed significantly reduced CD46 levels in infected mice, but normal CD46 amounts in peptide-P3 treated mice ($P \leq 0.05$) (**Fig. 6b and c**). The most likely explanation is that loss of CD46 occurs as a result of bacterial infection[28]. Cytokines are key molecules in induction of inflammatory responses and consequent cell damage. Therefore, we investigated the host response elicited by *H. pylori* and

measured IL-10, IL-6, and TNF-α, in the gastric mucosa. The level of IL-10 in P3-treated mice was higher than in water-treated mice, whereas IL-6 and TNF-α levels remained unaffected. The finding of increased level of the anti-inflammatory cytokine IL-10 of treated mice, suggests that P3 supplementation may reduce inflammatory responses (**Fig. 5d**). Taken together, our data demonstrate that peptide P3 has an *in vivo* bactericidal effect and indicate that supplementation of P3 might enhance anti-inflammatory responses in the host.

DISCUSSION

Gastric adenocarcinoma is strongly associated with the presence of *H. pylori*. One mechanism by which *H. pylori* may augment the risk of carcinogenesis is by altering cellular turnover. Research to date has shown that *H. pylori* has evolved numerous strategies to facilitate its persistence in the stomach including limiting the bactericidal effects of pro-inflammatory molecules and varying the antigenic repertoire of surface-exposed proteins. In this study we identify a new mechanism that may contribute to *H. pylori* pathogenesis. We show that *H. pylori* infection results in shedding of cell surface CD46 molecules from gastric cells and implicates CD46 as antimicrobial inhibitor for *H. pylori* growth and urease activity. We find that CD46 interacts with UreA and AhpC, two essential virulence factors of *H. pylori*. The bacterial binding occurred primarily within a short 24 amino acid peptide-domain at the C-terminal half of CD46. When this peptide was fed to *H. pylori* infected mice, it resulted in clearance of bacteria from the gastric mucosa, suggesting this peptide as a therapeutic agent.

It has been reported that a glycoprotein of CD43 containing ∝1,4-GlcNAc-capped O-glycan inhibit bacterial growth since it disrupts the biosynthesis of peptidoglycan in the cell wall of *H. pylori*[37]. Interaction between CD46 and bacteria has not previously been reported to reduce bacterial survival. We found that binding of CD46 led to reduced growth, membrane permeabilization, morphological abnormalities and death, suggesting that CD46 targets an essential molecule of the bacteria. By using affinity columns, UreA was pulled down from bacterial lysates. Urease is consistently present in all naturally occurring strains and mutants lacking UreA fail to colonize mice[10]. Indeed, the current study clearly implicates CD46 as inhibitor of urease activity, and earlier findings by Nagata *et al.* (1993)[38] suggested that inhibition of urease activity is

lethal to *H. pylori*. In addition to UreA, the affinity columns and mass spectrometry also identified AhpC. To combat the reactive oxygen species released by the host, *H. pylori* is equipped with a number of detoxifying proteins. The most abundant among these is AhpC, a thioredoxin-dependent member of the 2-Cys peroxi-redoxins[33], with higher homology to the human peroxiredoxins family Prxs than to eubacterial AhpC[39,34]. It is likely that binding of CD46 to AhpC affects the detoxification of organic peroxides and by this bacterial survival.

Infection of CD46 transgenic mice with *H. pylori* 67:21 resulted in colonization and reduction of CD46 at the gastric mucosa. However, not all strains are able to colonize; failure of J99 to establish infection in the mouse stomach indicates that a multitude of factors are involved in the bacteria-host cell interplay. The finding that *H. pylori* induces loss of CD46 in target cells is consistent with the finding that expression of CD46 in the gastric epithelium is weaker in patients with *H. pylori*-induced mucosal inflammation compared with uninfected individuals[36]. Eradication of bacteria by treatment with peptide P3 resulted in normal CD46 levels similar to wild-type mice, most likely due to the eradication of *H. pylori*. It is possible that shedding of CD46 could play an important role in innate defense to this pathogen and it remains to be analyzed if individuals with increased CD46 shedding are less prone to be colonized by *H. pylori*. Only a fraction of individuals infected with *H. pylori* develop inflammation and chronic gastritis. Taken into consideration the interaction between CD46-urease and CD46-AhpC, we can hypothesize that low CD46 amounts in the stomach may increase the risk of developing various diseases including peptic ulceration, gastric adenocarcinoma and gastric lymphoma.

The association between chronic inflammation and cancer is well established. There is mounting evidence suggesting a role of CD46 in the host immune system during bacterial infection and the up-regulation of CD46 expression in breast and other cancers has been indicated to correlate with carcinogenesis. Based on these reports, CD46 transgenic mice chronically infected with *H. pylori* may have an increased risk of developing gastric cancer. CD46 transgenic mice with *H. pylori* infection may represent a useful tool for investigating how the loss of CD46 contributes to carcinogenesis.

In conclusion, CD46 binds to *H. pylori* and inhibits urease, an enzyme essential for bacterial survival in the gastric environment. Docking studies suggest a mechanism wher CD46, and especially peptide P3, might effect either active sites of the urease or the functional region of AhpC The anti-bacterial spectrum of the P3-peptide is restricted to *H. pylori* and would therefore not disturb the normal gastrointestinal micro flora. In this respect, P3-peptide appears to possess, to some extent, the properties required of an effective and well-tolerated anti-ulcer agent. Further understanding of the interaction between CD46 and *H. pylori* UreA and AhpC provides a venue for developing novel treatments against *H. pylori* infection.

Methods

Bacterial strains and cells

H. pylori strains J99 (ATCC 700392) and 266995 (ATCC 700824) were from American Type Cell Collection. *H. pylori* HPGA1 and 67:21 have been described[40]. The bacteria were grown on Columbia blood agar plates (Becton Dickinson) supplemented with 8% horse blood and 8% horse serum at 37°C in a 10% CO_2 and 5% O_2 atmosphere. For liquid culture, bacteria were grown in Brucella broth (Becton Dickinson) containing 8% horse serum and 5ml/L IsoVitox enrichment (Dalynn Bilogicals). The human gastric cell line AGS (ATCC CRL-1739) were maintained in RPMI and 10% FCS.

Adherence assay

Bacteria (MOI=100) were allowed to adhere to AGS cells for 18 h, unbound bacteria were washed away and adhered bacteria were plated for viable counts. See Supplementary Methods for a detailed protocol.

Flow cytometry analysis of cells

CD46 expression on AGS cells: AGS cells were infected with *H. pylori* (MOI=100) for 18 h. Cells were collected, washed, and stained with polyclonal antibody to CD46 and Alexa488-conjugated anti-IgG antibody before analysis by flow cytometry (for detailed protocol see Supplementary Methods).

ELISA analysis

Cell supernatants: AGS cells cultured in 6 well plates were infected with *H. pylori* (MOI=100) for 18 h. Supernatants were collected, concentrated, re-suspended in 100

mM bicarbonate buffer (pH 9.5), and used to coat microtiter plates. CD46 was detected with polyclonal antibody against CD46 for 1 h followed by horseradish peroxidase (HRP)-conjugated goat anti-rabbit IgG as indicated in (Supplementary Methods).

Interaction between CD46 and H. pylori: Microtiter plates were coated with 10^7 CFU/ml of bacteria. One hundred µl of 2.5 µg/ml CD46 were added for 1 h. CD46 was detected with antibodies as above.

Purification of CD46 and affinity chromatography

Recombinant CD46 was expressed in *E. coli* and purified (see Supplementary Methods). Cell extracts of *H. pylori* J99 (10^8 bacteria/ml) was prepared as described previously[38], and loaded onto an Talon metal affinity column (Clontech) conjugated with recombinant CD46. Fractions were eluted, separated on SDS-PAGE, stained with Coomassie brilliant blue, and identified by mass spectroscopy.

Flow cytometry analysis of bacteria

CD46 and peptide binding to H. pylori: Bacteria at 10^7 CFU/ml were incubated with 30 µg/ml purified CD46 for 2 h, and then with polyclonal antibody to CD46 and Alexa488-conjugated anti-IgG antibody before flow cytometry analysis. To analyze binding of peptides, FITC-conjugated peptides (30 µM) were used incubated with bacteria for 2 h as above. See Supplementary Methods for a detailed protocol and peptide synthesis**.**

Competition: Purified CD46 of 30 µg/ml was pre-incubated for 1 h with 2.5 µg/ml of C3b (Calbiochem, CA, USA), or with 2 µg/ml of antibody to CD46 (H-294, Santa Cruz), or buffer alone. *H. pylori* was added at 10^7 CFU/ml for additional 2 h, whereafter the samples were fixed, stained with anti-CD46 antibody and analyzed by

flow cytometry. To evaluate bacterial lysis, CD46 (30 μg/ml) was pre-incubated with 2.5 μg/ml of C3b for 1 h, and followed by additional 2 h incubation with *H. pylori*. Bacteria were stained with PI according manufactures protocol (BD Biosciences) and analyzed by flow cytometry. The percentage of permeabilized cells was determined by subtracting the percentage of cells in the untreated population from percentage in the treated population.

Fluorescence microscopy.

30 μg/ml CD46 was incubated for 2 h with 10^6 CFU/ml *H. pylori* and stained according to the procedure for flow cytometry. Images were collected at 63x magnification using a fluorescent microscope (Zeiss). Images were further processed with Adobe Photoshop. For histological evaluation, the stomach tissues were fixed and embedded in paraffin. Intensity of signals was analyzed by Image J.

Survival assay.

Two hundred μl of 10^5 CFU/ml *H. pylori* suspension was incubated with 30 μg/ml CD46 for 0, 6 or 24 h or with 0-100 μg/ml CD46 for 6 h. Thioredoxin and PBS were used as control. Bacteria were serially diluted and plated on Columbia Blood Agar plates and incubated for 4-7 days before enumeration. *H. pylori* was incubated with 30 μM peptide P1, P2, P3 or P4, or incubated with different concentrations of the P3-peptide, and the growth of bacteria was monitored as above.

Construction of mutants, urease activity assay, and molecular docking.

See Supplementary Methods for protocols.

***In vivo* experiments.**

A CD46 receptor transgenic mouse strain (hCD46Ge) that expresses the CD46 receptor in a pattern closely mirroring that in humans was used[22,41]. Animal care and experiments were in accordance with institutional guidelines and have been approved by national ethical boards. Six to eight weeks old mice were administered orally with 0.1 ml of *H. pylori* 67:21 at 10^8 CFU/ml. At 8 weeks post infection, mice were orally fed with 100 µl 20 µM P3 or 100 µl of PBS once per day for 2 weeks. The mice were anesthetized and sacrificed for determination of viable bacteria in the stomach, immunohistochemistry, and cytokine expression see Supplementary Methods.

Supplementary Methods

Adherence of *H. pylori* to gastric epithelial cells. The human gastric cell line AGS (ATCC CRL-1739) was cultured in 24 well plates to 90% confluency. *H. pylori* strains were grown overnight in Brucella broth. Bacteria were washed twice with PBS, and re-suspended in cell medium. The medium of the AGS cells was removed and bacteria were added at a MOI (multiplicity of infection) of 100.. At 18 h post infection the cells were washed three times with PBS to remove unbound bacteria. The infected cells were then incubated with 1% saponin for 5 min and serial dilutions were spread on Columbia blood agar plates. Colony forming units were enumerated at 37 °C for 3-5 days after plating.

Flow cytometry analysis of cells. AGS cells were cultured in 6 well plates to 90% confluency. Cell medium was changed prior to infection with *H. pylori* (MOI 100) grown overnight in Brucella broth. At 18 h post infection, cells were collected, washed twice with PBS, and centrifuged for 5 min at 1000g. The pellets were re-suspended in PBS containing polyclonal anti-CD46 antibody (H-294, Saint Cruz, 200 μg/ml, diluted 1:200) and incubated 1 h on ice. Cells were washed in PBS and re-suspended in Alexa 488-conjugated anti-rabbit IgG antibody (Invitrogen) in PBS and incubated 1 h on ice. Cells were washed twice in PBS, fixed with 4% paraformaldehyde, washed and analyzed by flow cytometry. Data were processed by Cellquest Pro software (Becton Dickinson).

ELISA of cell culture supernatants. AGS cells were infected as above. Supernatants (10 ml) of infected and uninfected cells were collected after 18 h, concentrated, and re-suspended in 100 mM bicarbonate buffer (pH 9.5). Microtiter plates were

incubated over night at 4°C with 100 µl bacterial suspension per well. Wells were blocked with 5% skim milk for 2 h, washed with PBS, and incubated with polyclonal antibody against CD46 for 1 h (H-294, Santa Cruz, 200 µg/ml, diluted 1:5000). Incubation with primary antibody was followed by horseradish peroxidase (HRP)-conjugated goat anti-rabbit antibody (Invitrogen, 1:4000). After washing with PBS, substrate was added and absorbance at 450 nm was measured. Experiments were repeated twice in triplicate and the mean and standard deviation were calculated.

Purification of CD46.

The complete extracellular region of the CD46-BC1 isoform was PCR amplified with forward primer 5'-CACCTGTGAGGAGCCACCAACATT-3' and reverse primer 5'-ATCCAAACTGTCAAGTATTCCTTCCTC-3', and ligated into TOPO expression vector pET102D. The recombinant trx-CD46-6xhis protein was produced by BL21 (DE3) *E.coli* strain and purified with Talon metal affinity column (Clontech) according to the manual. Briefly, the cells were cultured for 4 to 6 h at 37°C in LB medium with 200 µg/ml ampicillin. At OD_{600}=0.6-0.8, the culture was induced by 0.5 mM IPTG for additional 4 h. Bacteria were harvested and incubated for 20 min RT in lysis buffer containing 50 mM Tris, 50 mM NaCl, 10 mM $MgCl_2$, 5 mM imidazole and lysozyme 5 mg/ml. The mixture were incubated in 0.1 mM PMSF and 1 U/ml DNase at RT for 5 min, sonicated and centrifuged at 15 000 g for 1 h. Protein was purified over a Talon resin column, and eluted with 200 mM imidazole. The thioredoxin control protein was produced by BL21 (DE3) host cell contain pET32a+ vector and purified. Protein purity was checked on an SDS-PAGE gel (**Supplementary Fig. 2a**).

ELISA of the interaction between CD46 and *H. pylori*. Bacteria from plates were suspended into Brucella broth containing 8% horse serum and 5ml/liter isovitox and harvested by centrifugation, washed three times with PBS, and re-suspended to 10^7 CFU/ml in 100 mM bicarbonate buffer, pH 9.5. Microtiter plates were incubated over night at 4°C with 100 µl bacterial suspension per well. Wells were blocked with 5% skim milk for 2 h, washed with PBS, and incubated with 100 µl of 2.5 µg/ml recombinant CD46 for 1 h, and with polyclonal antibody against CD46 for 1 h (H-294, Santa Cruz, 200 µg/ml, diluted 1:5000). Incubation with secondary antibody and substrate was done as above. Experiments were repeated twice in triplicate and the mean and standard deviation were calculated.

Generation of *H. pylori* ΔureA mutant

Chloramphenicol resistant gene (CAT) was PCR amplified from pACY184 using primers Cat1 (5′-CACTACGCTGGAAAATCAGTAAGTTGGCAGCATCACCCG-3′) and Cat2 (5′-TTTAGCACCATGCCAGGCGTTTAAGGGCACCAATAA′). *H. pylori* J99 genomic DNA was isolated from bacteria grown for 48 h by using Qiagen Genomic DNA kit as per manufacture's instructions. Two pairs of *ureA*-specific primers U1 (5′-TTGGCGCTGGGGTTTCTAATGT-3′), U2 (5′-ACTTATACTGATTTTCCAGCGTAGTGGAGCATCAACTT-3′) and U3 (5′-CTTAAACGCCTGGCATGGTGCTAAAAGCATGACAAC-3′), U4 (5′-CGCTTGCAAAAGCTGTAGGGAT-3′), were used to PCR amplify the up- and downstream regions, respectively. PCR products were purified using Qiagen Gel purification kit. The primers were designed with overlapping homologies at the ends. The three PCR products were combined into one fragment by five cycles without any primers followed by 30 cycles with two primers. The construct was introduced into *H. pylori* J99 by spot transformation. Bacteria grown for 48 h were suspended into 0.2

ml Brucella broth (Becton Dickinson) and spotted as 100 µl ($1x10^8$ bacteria/ml) onto Columbia blood agar plates. After incubation with 150 ng of purified DNA product, the bacterial transformation spot was spread over the plate and incubated for further 24 h. The bacteria were then moved onto selective Columbia blood agar plates containing 15 µg of chloramphenicol/ml and incubated for 3 to 7 days. Positive clones were confirmed by PCR, western blot and urease activity test.

Generation of *H. pylori* ΔureA/ΔaphC mutant

The alkyl hydroperoxide reductase gene was inactivated with a kanamycin resistance gene and used to transform the *ΔureA* deficient mutant of *H. pylori* J99. The kanamycin resistance gene was amplified from pDONR, P4-P1R with the primers *kan*-fwd (5'-GCCTGCCGTTTTGTATTAGTGACCTGTAGAATTCGAGC-3') and *kan*-rev (5'-ACCGCCTTGGTGTTAGAAAAACTCATCGAGCATCAAATG-3') The upstream region of the *ahpC* gene was amplified with the primers *ahpC*-ups-fwd (5'-ATCACTGCTCATGGGTTTAATGCG-3') and *ahpC*-ups-rev (5'-GGTCACTAATACAAAACGGCAGGCGCTTTAAAATCGG-3'). The downstream region of the gene was amplified with the primers *ahpC*-ds-fwd (5'-TGAGTTTTTCTAACACCAAGGCGTTGCAGAGTATCTT-3') and *ahpC*-ds-rev (5'-CATTTTTCTGTCCAAATTAAACCG-3'). The primers were designed to have overlapping ends. The three different PCR fragments were ligated in a PCR done in one step: (i) 7 cycles without any primers added followed by (ii) 35 cycles with the primers *ahpA*-ups-fwd and *ahpA*-ds-rev. The fragment obtained was incorporated into *ΔureA* deficient *H. pylori* as above by selective plate (10 µg/ml kanamycin and 10 µg/ml chloramphenicol). Positive transformants were confirmed by PCR using the primers *ahpC*-fwd (5'-CCACAAAGGTTACCACAAGATCAGG-3') and *ahpC*-rev (5'-CAGCAATCACTGAGCGTTTTTTAAGG-3').

Flow cytometry analysis of bacteria. *H. pylori* at 10^7 CFU/ml were pre-incubated with 30 μg/ml recombinant CD46 or 30 μM FITC-P3 peptide for 2 h at 37°C. Unbound CD46 was removed by washing with PBS. The pellets of bacteria incubated with CD46 were re-suspended in PBS containing polyclonal antibody against CD46 (H-294, Saint Cruz, 200 μg/ml diluted, 1:200) and incubated for 1 h on ice. Bacteria were washed in PBS and re-suspended in Alexa 488-conjugated anti-rabbit IgG antibody (Invitrogen, diluted 1:400) in PBS for 1 h on ice, washed twice in PBS, fixed with 4% paraformaldehyde, washed and analyzed by flow cytometry. Data were further processed by Cellquest Pro software (Becton Dickinson).

Peptide synthesis of CD46 domains. CD46 domains corresponding to the region (amino acids in relation to mature protein) 32-56 in CCPR-1, 81-105 in CCPR-2, 128-153 in CCPR-3, 190-221 in CCPR-4, FITC-32-56, in CCPR1, and FITC-128-153 in CCPR-3 were synthesized by Peptide 2.0, Inc (http://www.peptide20.com). All the peptides were 90-95% pure. Peptides were re-suspended in PBS and stored at -20°C prior to use. Peptides were subsequently diluted in Brucella broth or water to the working concentration.

Urease activity assay. The method to measure urease activity has previously been described[38]. Briefly, bacteria at 10^8 CFU/ml were centrifuged, washed and re-suspended with ice-cold de-gassed 20 mM sodium phosphate (pH 6.8). The suspension was sonicated for 3 min in 30 s pulses with ice cooling, centrifuged at 10 000 g for 20 min to remove cell debris. The supernatant was used for the urease activity assay. One μg of protein was incubated with 30 μg/ml of purified CD46 or peptides for 2 h at 37°C. After incubation, 100 μl of 50 mM phosphate buffer pH 6.8

containing 50 mM urea, 0.02% phenol red was added to each well. The color change was measured at 560 nm with micro-plate reader. Percentage inhibition was determined by the following equation: % inhibition = [activity without CD46] – [activity with CD46] / [activity without rC46] x 100.

Molecular docking. All experimentally determined structures used in this paper were downloaded from the Protein Data Bank (www.rscb.org). The three dimensional structure of *Helicobacter pylori* urease[42] (PDB ID: 1e9y or 1e9z), alkyl hydroperoxide-reductase AhpC[43] (PDB ID: 1ZOF), and the CD46[44] (PDB ID: XXXX) have been solved. Proteins were docked using the Zdock program[45] on a dense 6Å grid producing 54000 unique poses. Urease or AhpC chains were kept immobilized as receptor whereas CD46 changed its position on the grid. The generated poses were subsequently scored using the Zdock score which combines surface complementarity, electrostatic energy and potential based on statistics of interface atomic contacts. In sequential steps structures from the top of the ranking were clustered using Root Mean Square Deviation (RMSD) distance calculated for all heavy atom positions. All structures closer than 9.0Å RMSD were clustered together and outliers removed from pool. This ranking based clustering allows for a relatively fast extraction of the most representative poses. Contacts within 4Å were recorded for all the representative structures. Interacting interfaces of selected poses were analyzed by the PIC[46] web service, which summarizes all important interactions in complex interface.

***In vivo* experiments.** A CD46 receptor transgenic mouse strain (hCD46Ge) that expresses the CD46 receptor in a pattern closely mirroring that in humans was

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Deleted: . CD46 residues involved were grouped according to specificity of its backbone and side chain milieu. Frequencies of contacts of all ligand poses generated in docking are additionally calculated to reflect difference between single pose contacts and general tendency in large molecule ensemble.

used[22,41,47]. Animal care and experiments were in accordance with institutional guidelines and have been approved by national ethical boards. Six to eight weeks old mice (n=24) were administered orally with 0.1 ml of *H. pylori* 67:21 at 10^8 CFU/ml once per day for 3 days interval. Uninfected control mice (n=10) were dosed with 0.1 ml of PBS in a similar way. At 8 weeks post infection, infected mice were divided into two groups; one group was inoculated orally with 100 µl 20 µM P3-peptide, the other group with 100 µl of PBS, once per day for 2 weeks. All mice were anesthetized and sacrificed two weeks after treatment. The abdomen was opened by a midline incision and the stomach was isolated and cut along the greater curvature. The gastric content was gently washed out with saline. Two longitudinal strips with a width of 3–5 mm were taken: one for *H. pylori* culture and another for histological evaluation. Stomach tissues were homogenized in Brucella broth, colonization was determined by plating dilutions on blood agar plates containing (200 µg/ml) and nalidixic acid (10 µg/ml). Plates were incubated for 4–7 days and *H. pylori* colonies were identified by their morphology and urease production. For histological evaluation, the stomach tissues were fixed in 4% paraformaldehyde and embedded in paraffin. For detection of *H. pylori* and CD46, the tissue sections were incubated with mouse antibody against *H. pylori* (sc-65454, diluted 1:50) and rabbit antibody against CD46 (H94, Santa Cruz, diluted 1:50) for 1 h, followed by Alexa 488 anti-mouse IgG and Alexa 593 conjugated goat anti-rabbit IgG (diluted 1:50; Invitrogen) as a secondary antibodies. Sections were visualized with Carl Zeiss Axio Axiovision 2.05 image processing and analysis system (Zeiss).

IL-6, IL-10 and TNF-α assays.

The mouse stomach was homogenized in Brucella broth, and supernatants were collected in aliquots at -80 °C. The levels of IL-6, IL-10 and TNF-α were measured

using ELISA kits (Invitrogen) following manufacture's instructions. Each sample was measured in duplicates.

Statistical analysis. Experiments were performed in multiple replicates and the combined data for each experiment was analyzed by one-way ANOVA analysis (Graphpad Prism 4) to compare within the groups. P values <0.05 were considered statistically significant. For mouse infections, the results represent two independent experiments.

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AUTHOR CONTRIBUTIONS

R.BW. designed, performed, analyzed most of the experiments, an wrote the manuscript. H.S contributed with design, performance and analyzsis of *in vivo* experiments, and manuscript preparation. W.J and A.E. designed, performed and analyzed the molecular docking. N.G. constructed recombinant CD46, and performed CD46 binding and killing experiments. M.H., performed cytokine analysis. H.K and E.W. conducted *H. pylori* mutants. T.B., L.E. and A.A. provided *H. pylori* strains, consultation and suggestion of experiments and manuscript preparation. H.A. contributed to experimental design, participated in CD46 binding and killing studies, and manuscript preparation. AB.J. conceived, designed the study, analyzed the data, and wrote the manuscript.

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Figure Legends

Figure 1

Infection of gastric epithelial cells triggers shedding of human CD46. **(a)** AGS epithelial cells were infected with *H. pylori* strains J99, HPGA1, or 26695 at a MOI of 100. At 18 h post infection, cells were treated with 1% saponin, serial diluted, plated on blood agar plates, and enumerated for CFUs after 3-5 days growth. Shown are mean±SD based on triplicate samples of three independent experiments. **(b)** Flow cytometry analysis of CD46 expression in uninfected (grey area) and *H. pylori* infected (white area) AGS cells for 18 h (MOI=100). **(c)** ELISA analysis of supernatants from AGS cells infected with *H. pylori* strains for 18 h. Microtiter plates were coated with supernatants of infected and uninfected cells, and overlaid with a polyclonal antibody to CD46 followed by HRP-conjugated anti-IgG.

Figure 2

Interaction between CD46 and *H. pylori*. (**a**) Schematic drawing of the extra-cellular region of CD46-BC1 adapted from Riley-Vargas *et al*., 2004[17,48]. (**b**) Microtiter plate assay demonstrating binding of recombinant CD46, composed of extracellular CD46-BC1, to *H. pylori*. Microtiter plates were coated with 10^7 CFU/ml of *H. pylori* strains J99, 26695 and HPGA1, and overlaid with 2.5 µg/well CD46 for 1 h. Bound CD46 was detected by antibody to CD46 and HRP-conjugated anti-rabbit IgG. (**c**) Flow cytometry analysis of CD46-binding to bacterial strains. Bacteria at 10^6 CFU/ml were incubated with 30 µg/ml CD46 for 2 h, washed, stained with antibody to CD46 and Alexa488-conjugated anti-rabbit IgG before analysis by flow cytometry. (**d**) The interaction between *H. pylori* and CD46 is blocked by antibody to CD46 or C3b.

Purified CD46 was pre-incubated with buffer, C3b or CD46 antibody for 1 h at 37^{o}C, and then incubated for additional 2 h with *H. pylori* J99. Bacteria were washed, stained with antibody to CD46 and Alexa488-conjugated anti-rabbit IgG. (**e**) Microscopic detection of CD46-binding to *H. pylori.* Bacteria at 10^6 CFU/ml were incubated without (-CD46) or with (+CD46) 30 µg/ml of CD46 for 2 h, washed, stained with polyclonal CD46 antibody and anti-IgG antibody before analysis by fluorescence microscopy. Images are representative of one of three separate experiments.

Figure 3

Recombinant CD46 is bactericidal against *H. pylori*. Strain J99 (10^5 CFU/ml) was incubated with (**a**) 0, 5, 10, 30, 50 or 100 µg/ml purified CD46 (Trx-CD46) or purified thioredoxin (Trx) at 37 °C in 10% CO_2 and 5% O_2 for 6 h, or (**b**) 30 µg/ml of CD46 for 0, 6, and 24 h. Viable bacteria were enumerated by plating serial dilutions on Colombia Blood Agar plates. Bacterial survival is expressed as colony-forming units (CFU) after incubation with CD46 divided by CFU after incubation with buffer alone. (**c**) Permeabilization of bacteria by CD46 is blocked by C3b. CD46 was pre-incubated with buffer or C3b before addition to *H. pylori* strain J99 for 2 h. Purified thioredoxin (Trx) or buffer without CD46 were used as negative controls. Bacteria were stained with PI, fixed, and analyzed by flow cytometry. **(d**) Identification CD46-binding ligands of *H. pylori* by affinity chromatography. Two significant bands with molecular weights of 26 kDa and 21 kDa were identified as UreA and alkyl hydroperoxide reductase (AhpC) by mass spectrometry. (**e**) Flow cytometry analysis of CD46-binding to *H. pylori* J99 and its *ΔureA* and *ΔureA/ΔahpC* mutants. Bacteria at 10^6 CFU/ml were incubated with 30 µg/ml CD46 for 2 h, washed, stained with

antibody to CD46 followed by Alexa 488 conjugated anti-rabbit IgG before analysis by flow cytometry. (**f**) Time course inhibition of *H. pylori* urease activity by CD46. Urease activity was assayed by adding urea-containing reaction buffer as indicated in methods.

Figure 4

Mapping the *H. pylori*-binding region of CD46.

(**a**) Schematic drawing of the four CCPR-domains of CD46. Indicated are positions of four synthesized peptides, P1-P4, and their amino acid sequence. The amino acid positions are shown in parenthesis. **(b)** Survival of *H. pylori* after incubation with the CD46-derived peptides. Bacteria at 10^5 CFU/ml were incubated with 30 μM of each peptide for 6 h, serially diluted and spread onto plates for viable counts. (**c**) Survival of 10^5 CFU/ml *H. pylori* after incubation for 6 h with 0, 5, 10, 20 or 30 μM of the P3-peptide. Bacteria were serially diluted and spread onto plates for viable counts. Results represent three independent experiments each done in triplicates and error bars represent means ±SD.(**d**) Flow cytometry analysis of peptide binding to *H. pylori* J99 and its *ΔureA* and *ΔureA/ΔahpC* mutants. Bacteria (10^7 CFU/ml) were incubated with 20 μM of FITC-conjugated P3, P1 or random peptide (RP) for 2 h, washed, and analyzed by flow cytometry. (**e**) The effect of the P3 and P1-peptide upon urease activity of *H. pylori* J99. One μg of total protein extract of *H. pylori* J99 was incubated with 30 μM of P3 or P1 peptide for 0 to 60 min. Urease activity was assayed by adding urea-containing reaction buffer as indicated in methods. Results represent triplicate of three independent experiments each done in triplicates and error bars represent means ±SD.

Figure 5

Model of the CD46 interaction with *H. pylori* urease and AhpC. (**a**) Complete functional assembly of *H. pylori* urease according to Ha *et al*., 2001[42]. Catalytic beta subunits are shown in **cyan** and alpha subunits in **green**. (**b**) CD46 domains[44] CCPR3-4 docked to urease inter-segment hole consisting of two alpha subunits and two beta subunits showing that CCPR3 fits in the transport hole. **(c)** and **(d)** top and side view of CCPR3-4 docked to the urease transport hole having contacts with catalytic beta subunit. Urease is displayed as molecular surface with highlighted oxygen (**red**) and nitrogen (**blue**) atoms. CD46 is presented as wire with selected peptides displayed as sticks, P3-peptide (**pink**) and P4-peptide (**violet**). Only CCPR3 takes part in complex creation. Polar contacts together with molecular fit of two molecules play major role in complex stabilization. **(e)** *H. pylori* alkyl hydroperoxide reductase (AhpC)[43]. **(f)** Complete functional assembly with docked CD46. **(g)** Close up of CD46 domains CCPR3-4 docked to AhpC functional dimer. CD46 is presented as wire with selected peptides displayed as sticks, P3 (**pink**) and P4 (**violet**). In this case mainly CCPR takes part in complex creation.

Figure 6

Oral administration of P3-peptide eradicates *H. pylori* infection in a mouse model. CD46 transgenic mice, 10-12 mice per group, were infected orally with 10^8 CFU/ml of *H. pylori* strain 67:21 once per day for 3 days. At 8 weeks post-infection, mice were fed orally with either 20 μM P3-peptide in water (+P3) or water alone (-P3) for 2 weeks. Then mouse stomach tissue was collected and homogenized for bacterial enumeration on plates or prepared for immunohistochemistry. (**a**) CFU/g of bacteria in stomach of mice, shown as $\log_{10}$, of uninfected mice (control ●), mice fed with

water alone (-P3 ◆), mice fed with peptide P3 (+P3 ○). (**b**) Immunostaining of CD46 and *H. pylori* in stomach tissue at 10 weeks post infection using antibodies against CD46 and *H. pylori*. The level of CD46 in gastric tissues of *H. pylori* infected mice was significantly reduced compared to P3-treated mice ($P<0.01$). (**c**) Immunostaining of CD46 and *H. pylori* in gastric tissue sections at 10 weeks post infection using either anti-CD46 antibody or anti-*H. pylori* antibody. (**d**) Level of IL-10, IL-6, and TNF in gastric tissue measured by ELISA. The IL-10 level was elevated in P3-treated mice compared to water-treated mice (-P3). There was no significant difference in IL-6 and TNF levels between treated and untreated mice. Shown are means ±SD.

Supplementary figure legends

Supplementary Figure 1. Infection of AGS cells by *H. pylori* triggers cell death. **(a)** AGS cells were collected, lysed, subjected to 12% SDS-PAGE, and transferred to PVDF membranes. CD46 was identified with polyclonal antibody to CD46. CD46 migrates as a characteristic two-band pattern with molecular masses of 59–68 kDa and 51–58 kDa, representing the BC isoforms and the C isoforms, respectively. **(b**) Flow cytometry of PI-stained AGS cells. AGS cells were infected with *H. pylori* strains J99, 26695 and HPAG1 for 18 h. After infection the cells were harvested, washed, stained with propidium iodide (PI) according to manufacturers recommendations (BD Biosciences), and analyzed by flow cytometry. Uninfected control cells are marked in grey and *H. pylori* infected cells in white.

Supplementary Figure 2. CD46 does not bind to *C. jejuni* and *P. aeruginosa*. **(a)** Purified CD46 was separated on 12% SDS-PAGE and stained with Coomassie brilliant blue. Shown are molecular weight marker (Mw), CD46-thioredoxin fusion protein (CD46) and thioredoxin (Trx). **(b)** Flow cytometry analysis of CD46-binding to *C. jejuni* and *P. aeruginosa*. Bacterial strains (10^7 CFU/ml) were incubated with 30 µg/ml CD46 for 2 h, washed, stained with polyclonal antibody to CD46 followed by Alexa 488 conjugated anti-IgG. **(c)** Flow cytometry analysis of CD46-binding to *H. pylori* in medium at different pH. Bacteria (10^7 CFU/ml) were incubated with 30 µg/ml CD46 for 2 h in Brucella broth with pH 2, 4, or 7, washed, and stained as above.

Supplementary Figure 3. Binding of CD46 to *H. pylori* 67:21 and the effect of peptide P3 on bacterial survival. **(a)** Flow cytometry analysis of CD46-binding to *H. pylori* 67:21. Bacterial strains (10^7 CFU/ml) were incubated with 30 µg/ml CD46 for 2 h, washed, stained with antibody to CD46 followed by Alexa 488 conjugated anti-IgG. **(b)** Survival of *H. pylori* 67:21 after incubation with the peptide P3. Bacteria at 10^5 CFU/ml were incubated with 30 µM of peptide (P3) or buffer (C) for 6 h, serially diluted and spread onto plates for viable counts. Shown are a representative experiment out of three independent experiments and error bars show means ±SD.

Supplementary Table 1.

Amino acids of CD46 in contact with the Urease or AhpC.

Data	**Urease alpha**	**Urease beta**	**Urease assembly**	**AhpC**
CD 46, CCP3-4 amino acids in contact	Thr129 Pro130 Phe141 **Glu143 Glu145 Val146 Phe147 Tyr213 Tyr214 Lys215**	Pro165 Phe166 **Ser167 Arg195 Phe196** Pro197 **Val198 Val199 Lys203 Ile205** Phe208 **Lys210** Lys211 Lys248	**Leu127** Cys128 Pro132 **Lys133 Lys135 Glu143** Val144 **Glu145 Phe147 Glu148 Tyr149 Asp151** Ala152 Val153 **Thr154 Glu171 Asp177 Asn179 Arg184** Glu188 Phe212 **Tyr213**	Asp164 Pro165 **Phe166** Leu168 Lys173 Cys194 **Phe196 Pro197** Val198 **Val199 Asn201 Ile205 Phe208** Gly209 **Lys210** Lys211 Tyr214 **Asp223** Cys236 Ser238 **Trp242**

Hydrophobic interactions, ionic interactions and hydrogen bond donors and acceptors are all counted. In bold are residues having at least two contacts with either Urease or AhpC of *H. pylori*. Peptide P3 is predominantly interacting with Urease alpha and Urease assembly hole whereas both P3 and P4 are involved in AhpC and Urease beta complexes. High number of electron pair donor and acceptors within required geometrical constrains suggests importance of hydrogen bonding contribution and found in Urease assembly hole and Urease beta complexes (50 and 22 potential hydrogen bonds respectively).