Histamine signaling through the H_2 receptor in the Peyer's patch is important for controlling *Yersinia enterocolitica* infection

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Enteric pathogens such as *Yersinia enterocolitica* readily colonize and induce disease within the lymphatic tissues of the small intestine. To gain a comprehensive view of the host response to pathogens within these tissues, we determined the transcriptional profiles of intestinal lymphatic tissue infected with *Y. enterocolitica*. Expression analysis using Affymetrix GeneChips revealed a complex host response in the Peyer's patches and mesenteric lymph nodes after oral infection with *Y. enterocolitica*. Interestingly, histidine decarboxylase (*Hdc*) was significantly up-regulated in response to *Y. enterocolitica* infection. HDC is the enzyme solely responsible for the production of the biogenic amine histamine. Although histamine is well known for its role in allergy and for its effects on immunity and inflammation, little is known about its role or specific histamine receptors during the host response to bacterial infection. In this study, we provide evidence that histamine signaling through the histamine H_2 but not the H_1 receptor is important for controlling *Y. enterocolitica* infection within the Peyer's patches and mesenteric lymph nodes of mice.

IL-10 | IL-17 | IL-11 | inflammation

The intestinal immune system is composed of specialized lymphatic compartments, most prominently the Peyer's patches (PPs), mesenteric lymph nodes (MLNs), small isolated lymphoid follicles, and a diverse intraepithelial lymphocyte population (1). PPs are thought to be the primary inducers of the immune response to pathogens. Paradoxically, they are also a common site for colonization by viral and bacterial pathogens.

Several Gram-negative pathogens, such as those from the genera *Yersinia* and *Salmonella* are extremely successful at crossing the small-intestinal epithelium and colonizing the underlying lymphoid tissues (2–4). The ability of these bacteria to survive within these tissues is partially due to their immunomodulatory capacity implemented through the action of specialized bacterial proteins. The sophistication of the bacterial protein network involved in modifying the host response highlights the complexity of the immune response mounted in response to such pathogens. For example, in cell culture models, *Yersinia enterocolitica* can disable the phagocytic capabilities of macrophages and polymorphonuclear leukocytes (PMNs) and induce macrophage apoptosis. *Y. enterocolitica* also inhibits the normal operation of NF-κB and several other components of the inflammatory cascade resulting in the inhibition of proinflammatory cytokine production (TNF-α and IL-8), synthesis of monocyte chemoattractant protein 1, and reduced presentation of adhesion molecules (reviewed in ref. 5). Strains of *Y. enterocolitica* lacking the genes encoding these immunomodulatory proteins are deficient in their ability to colonize the PPs (6).

Although the above effects have successfully been studied in cell culture models, the roles they play during infection have yet to be completely elucidated. Dissimilarity between the results of cell culture experiments and *in vivo* experiments on intact tissues has been reported and highlights the importance of local environments in the host response to pathogens (7). Furthermore, animals infected with *Y. enterocolitica* still exhibit extensive pathology within colonized tissues including PMN and macrophage accumulation, granuloma formation, and necrosis, suggesting a host response even in the presence of such bacterial factors (8–10). Therefore, alternative approaches to studying the relationships between pathogen and host *in vivo* are merited to fully understand bacterial-induced intestinal disease.

In the mouse, *Y. enterocolitica*-induced disease is characterized by a rapid colonization and destruction of the PPs followed by colonization of the MLNs. From these sites of infection, the bacteria may spread to other tissues such as the spleen and liver. Previous studies have suggested roles for PMNs, macrophages, T_H1 T cells, and the cytokines TNF-α, IFN-γ, IL-1α/β, IL-1 receptor antagonist, IL-6, IL-12, and IL-18 within the PPs and/or MLNs (4, 11–16). Furthermore, several studies monitoring the transcriptional response of various cell lines to *Y. enterocolitica* have implicated these inflammatory genes in response to infection (17–19). However, a comprehensive survey of the host response in the PPs and MLNs has yet to be conducted.

A review of the literature reveals a large body of information concerning the regulation of interleukins and chemokines in the liver and spleens of mice infected either i.v. or i.p. with *Y. enterocolitica* (7, 13, 20–22). In contrast, little is known about regulation of immune response genes in tissues of the mucosal immune system after oral infection. Although several reports have shown intricate regulation of IL-1α, IL-1β, and IL-1 receptor antagonist in the PPs after oral infection, the regulation of other immune response genes has yet to be explored (11, 15, 16, 23). Not surprisingly, even less is known about the role of nonprotein-signaling molecules in the host response to *Y. enterocolitica* or indeed to other bacterial pathogens. Because pathogen-induced responses frequently result in changes in gene transcription, we used GeneChip technology to investigate the host response within intestinal lymphoid tissues after oral infection (24). This expression analysis revealed a complex response to *Y. enterocolitica* within the PPs and MLNs. To confirm that the data obtained from the GeneChip analysis could be used to define unique elements of the intestinal immune response, we selected a single gene that had increased transcript levels throughout the course of infection for further analysis. This gene encodes the enzyme histidine decarboxylase (HDC), which is exclusively responsible for the production of histamine. Using a pharmacological approach, we provide evidence that histamine signaling through histamine receptor 2 (H_2) is important for the early stages of

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Abbreviations: PPs, Peyer's patches; MLNs, mesenteric lymph nodes; PMNs, polymorphonuclear leukocytes; HDC, histidine decarboxylase; H_n, histamine receptor *n*; PI, postinfection; qRT-PCR, quantitative RT-PCR; PPI, proton pump inhibitor; cfu, colony-forming units; GO, gene ontology.

Data deposition: The data reported in this paper have been deposited in the Gene Expression Omnibus (GEO) database, www.ncbi.nlm.nih.gov/geo (accessible through GEO series accession no. GSE4764).

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controlling *Y. enterocolitica* colonization within the PPs of the small intestine.

Results

Transcriptional Profiling of Infected PPs and MLNs. We investigated the host response mounted in the intestinal lymphatic system in response to colonization with a bacterial pathogen. To perform this investigation, mice were orally infected with *Y. enterocolitica* and transcriptional responses in infected PPs and MLNs were analyzed at several time points postinfection (PI) by using Affymetrix GeneChips. By using the filtering techniques described in *Materials and Methods*, 421 genes (3.39%) from the PPs and 566 (4.56%) genes from the MLNs passed the threshold criteria to be included as having an increased or decreased number of transcripts after infection with *Y. enterocolitica* (see Fig. 4 *A* and *B*, which is published as supporting information on the PNAS web site). It is not possible to discern whether an increase or decrease in transcript number is the result of a gene regulatory event or due to some other effect such as cell influx, cell efflux, or cell death. Either case is biologically interesting, but for simplicity purposes from this point forward an increase in transcript number will be called "up-regulated" and a decrease "down-regulated." Details on individual transcripts and their expression patterns can be found in Tables 3 and 4, which are published as supporting information on the PNAS web site, for PPs and MLNs, respectively. As expected, a variety of cytokines, chemokines, and chemokine receptors were differentially expressed during infection, including some previously unidentified molecules (Table 1). Cytokine regulation in response to *Y. enterocolitica* included increases in IL-11 and IL-17 in the PPs and the MLNs. This observation was further confirmed by quantitative RT-PCR (qRT-PCR) (Fig. 1*A* and see Fig. 5, which is published as supporting information on the PNAS web site). Also of note is that cytokines IL-2, -4, and -10, previously implicated by some studies, did not show a change in transcript in our study at any time point in either the PPs or MLNs (20, 25, 26).

Production of Histamine in PPs After Oral *Y. enterocolitica* Infection. One of the unexpected and previously unknown finding from this analysis was the up-regulation of histidine decarboxylase (*Hdc*) in response to infection. These data implicate histamine in the host response to *Y. enterocolitica* infections because HDC is the enzyme solely responsible for production of the biogenic amine histamine. To confirm this transcriptional regulation and determine whether the increase in transcript correlated with an increase in biological activity, we performed confirmatory qRT-PCR analysis and histamine immunohistochemical studies. Transcription of the gene-encoding HDC was up-regulated in both PPs and MLNs after oral *Y. enterocolitica* infection (Fig. 1*A* and see Fig. 5). To determine whether the transcriptional up-regulation of *Hdc* correlated with increased histamine production, immunohistochemistry using an anti-histamine antibody was performed. After oral inoculation with PBS, little to no staining for histamine was seen in the PPs (Fig. 1*B*). However, after inoculation with *Y. enterocolitica*, robust histamine immunoreactivity was detected within the PPs. The staining was punctate, surrounded by a more diffusely stained halo. Based on the nuclear staining, histamine production was also occurring in a region of dead or dying cells, suggesting that this area is an actively infected region within the PPs. The fixation process required for the anti-histamine staining is not compatible with staining with our anti-*Yersinia* antibody. However, previous analyses of sections from infected mice for the presence of *Y. enterocolitica* indicate that they would be found at these sites (10, 27, 28). This finding implies that histamine is produced by a specific subset of the PPs cells in close proximity to the infected area. Therefore, histamine production correlates with the increased *Hdc* transcript.

Table 1. Regulation of host immunomodulatory proteins

Immune molecule	Average signal-log ratio: Day PI/PP 2	Day PI/PP 4	Day PI/MLN 2	Day PI/MLN 4
Known				
IL-1α	3.5	2.6	2.1	1.1
IL-1β	5.6	5.3	6.2	4.6
IL-1Ra	2.7	1.9	3.2	1.2
IL-6	3.7	3.5	4.6	3.8
IL-12 p35	1.6	1.0	0.16	3.5
IL-12 p40	—	—	2.7	1.8
IL-18	—	—	0.3	0.6
TNF-α	2.4	2.4	—	—
IFN-γ	1.0	4.9	—	—
CXCL2	4.3	4.8	4.8	2.7
CXCL10	3.0	2.4	3.0	1.3
CCL2	4.8	5.0	3.2	0.8
CCL3	2.1	2.3	1.6	0.5
CCL4	2.5	1.9	2.8	−0.2
Unknown				
IL-11	3.6	3.1	—	—
IL-17	3.0	3.4	2.2	2.0
CXCL1	3.8	3.9	2.8	2.3
CXCL5	3.4	4.2	2.1	1.2
CXCL9	3.0	2.9	3.7	1.9
CXCL10	3.0	2.4	3.0	1.3
CCL7	5.6	5.4	5.0	3.0
CX3C1	1.3	−0.1	—	—
CCL8	—	—	1.9	1.4
CCL9	—	—	1.0	1.8
CCL12	—	—	4.2	2.5
CCR5	2.6	3.1	3.1	2.4
CCR1	—	—	2.1	2.0
CCR2	—	—	1.7	1.6

Average signal-log ratios were determined as described in *Materials and Methods* for samples from mice orally infected with $\approx 5 \times 10^8$ *Y. enterocolitica* in comparison with mice orally infected with PBS. The following previously implicated immune response molecules did not show a change in amount of transcript in our study at any time point in either the PP or MLN: IL-2, IL-4, IL-10, CXR2, CXCR4, and CXCR5. —, indicates that there was no change in the amount of transcript at the indicated time point/tissue.

Signaling Through H_2 Is Important for Controlling *Y. enterocolitica* Infection. Although a great deal of research has been done on the role of histamine in allergy, immunity, and inflammation (29), little is known about the role of specific histamine receptors during the host response to bacterial infection. Histamine exerts its biological effects by interacting with receptors on target cells; four histamine receptors are known (H_1, H_2, H_3, and H_4), and it is the specific histamine receptor interaction as well as the properties of the cell expressing the receptor that determines the biological outcome of the interaction (30–35). To elucidate the role of histamine during *Y. enterocolitica* infection, we pharmacologically antagonized or agonized individual histamine receptors that have been implicated as being involved in various aspects of the immune response. A pharmacological approach was chosen rather than using knockout mice because of the availability of highly specific reagents and because the knockout mice could have pleiotropic developmental effects on the immune system. In addition, we focused on H_1 and H_2 because, compared with H_3 and H_4, they have a broad distribution on immunological relevant cells and because highly specific reagents are available that have been used in *in vivo* studies.

As a control before these studies, growth curves were established

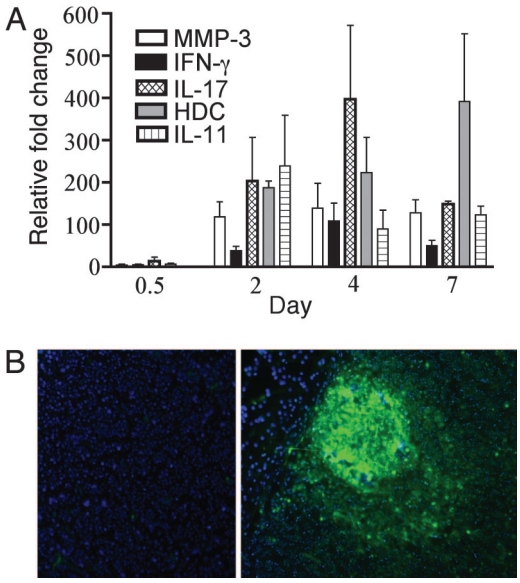

Fig. 1. Independent confirmation of the host response to *Y. enterocolitica* infection. Transcripts of interest, shown to be up-regulated in the PPs in response to *Y. enterocolitica* infection by GeneChip analysis, were selected for confirmatory qRT-PCR analysis. Each column represents three values obtained from qRT-PCR amplification of cDNAs generated from three different groups of five mice each. qRT-PCR results from infected PPs (*A*) and immunohistochemical visualization of histamine production in PPs (*B*). (*B*) Tissues from uninfected mice (*Left*) or mice 2 days PI with *Y. enterocolitica* (*Right*). Sections were stained for histamine (green) and nuclei (blue).

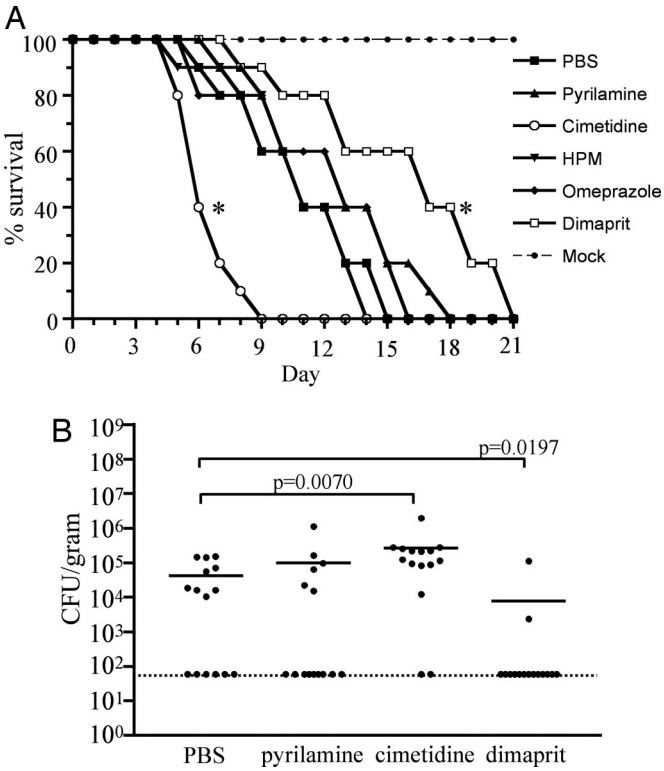

Fig. 2. Effect of treatment with histamine receptor antagonists and agonist on infection with *Y. enterocolitica*. (*A*) Survival of mice after oral *Y. enterocolitica* infection (5–8 $\times$ 10^8 cfu) with or without i.p. administration of PBS, pyrilamine, cimetidine, dimaprit, or oral administration of the PPI omeprazole or the omeprazole diluent (hydroxypropyl)methyl cellulose (HPM). Mock represents the survival of mice treated daily with the drugs in the absence of infection. The experiment was repeated using 10 mice per treatment group and resulted in similar survival curves. Data from only one of the two experiments is displayed. An asterisk indicates that the survival curve is significantly different from the survival curve generated from PBS-treated mice (cimetidine, $P = 0.0020$; dimaprit, $P = 0.0076$). (*B*) Colonization of MLNs after oral infection with *Y. enterocolitica*. Mice were treated with 6–8 $\times$ 10^8 cfu after i.p. injection with PBS, pyrilamine, cimetidine, or dimaprit. Bacterial colonization within the MLNs was determined 3 days PI. Three independent experiments were done by using five mice per treatment and combined into a single set of data. The average limit of detection (indicated with a dotted line) is 60 bacteria. *P* values were determined by comparing treatments with each agonist or antagonist with PBS treatment by using the Mann–Whitney test.

with the following concentrations of each drug: pyrilamine, 0.1 and 1.0 mg·ml^{-1}; cimetidine, 0.1 and 1.0 mg·ml^{-1}; and dimaprit, 0.1, 1.0, and 5.0 mg·ml^{-1}. No difference in the growth curve compared with LB alone over 15 h (well into stationary phase) was evident, although the concentrations used were higher than what would be expected in the mouse (data not shown).

H_1 receptor antagonism with pyrilamine had no statistically significant effect on the survival of infected animals compared with mice treated with PBS (Fig. 2*A*). Uninfected mice treated daily with pyrilamine survived the duration of the experiment and never demonstrated any obvious physiological or histological changes in their PPs (data not shown). Bacterial numbers in the lumen of the small intestine at days 2 and 5 PI or within the stomach at day 1 PI neither were significantly different between PBS- and pyrilamine-treated mice (data not shown) nor were the numbers of colonized PPs and the level of bacterial dissemination to the MLNs (Table 2 and Fig. 2*B*). These data suggest signaling through H_1 has no effect on bacterial colonization of individual tissues or overall survival after oral *Y. enterocolitica* infection.

In contrast, treating mice with the H_2 antagonist cimetidine resulted in a decrease in survival after *Y. enterocolitica* infection compared with mice treated with PBS (Fig. 2*A*). Treatment of mice with cimetidine alone neither had an effect on the survival of the mice nor induced any obvious physiological or histological changes in their PPs (data not shown). The PPs of cimetidine-treated mice infected with *Y. enterocolitica* became colonized earlier than mice treated with PBS (Table 2). Bacterial dissemination from the intestine to the MLNs occurred at a higher frequency in cimetidine-treated mice than in PBS treated mice (Fig. 2*B*). MLNs colonized with bacteria also had higher overall bacterial loads per gram of tissue in mice treated with cimetidine compared with mice treated with PBS (Fig. 2*B*). Colonization of the lumen of the stomach and small intestine was similar between PBS and cimetidine-treated mice at day 2 PI (see Fig. 6, which is published as supporting information on the PNAS web site, and data not shown).

On closer examination of the small intestines, cimetidine-treated mice also exhibited a high frequency of intussusception compared with either PBS or H_1 antagonist-treated mice. Intussusception occurs when one portion of the intestine slides into the next, creating an obstruction that can lead to swelling, inflammation, and decreased blood flow. In general, the intussusception frequently occurred in an area of the intestine containing a PP, suggesting a correlation to infection. However, due to extensive tissue damage at this site, it was sometimes difficult to anatomically determine whether a PP was located in a specific portion of the intestine before infection. Intussusception eventually occurred in 20% of PBS-treated mice infected with *Y. enterocolitica* but typically not before day 10 PI; in contrast, 60% of cimetidine-treated mice infected with *Y. enterocolitica* showed intussusception by day 6 PI. None of the mice treated with dimaprit had identifiable intussusception after being infected for 14 days. Treatment of infected mice with the H_2 agonist dimaprit increased mouse survival after *Y. enterocolitica* infection and decreased the colonization of PPs and MLNs during the early stages of infection (Fig. 2 *A* and *B* and Table 2). Colonization of the lumen of the small intestine was equivalent in

Table 2. Effect of drug treatment on the colonization of PP with *Y. enterocolitica*

	% positive for *Y. enterocolitica* antibody					
Day/PI	PBS	Pyrilamine	Cimetidine	Dimaprit	Omeprazole	HPM
1	0.0%	7.1%	40.0%	0.0%	0.0%	0.0%
	0/0 (38)	1/2 (42)	9/7 (40)*	0/0 (42)	0/0 (36)	0/0 (43)
3	29.2%	32.6%	59.0%	13.9%	25.0%	25.0%
	9/5 (48)	7/8 (46)	13/10 (39)*	2/3 (36)	4/8 (48)	6/4 (40)

Colonization of PP after oral inoculation with $\approx 5 \times 10^8$ *Y. enterocolitica* and i.p. administration of pyrilamine, cimetidine, dimaprit, or oral administration of omeprazole or (hydroxypropyl)methyl cellulose. Sections were called positive if *Y. enterocolitica* were visible under the ×40 objective after staining with anti-*Yersinia* antibody. Numbers obtained from two independent experiments using groups of five mice per treatment (no. of PP positive in experiment 1/no. of PP positive in experiment 2). Numbers in parenthesis indicates the total number of PP examined per treatment. An average of four PP per mouse were identified and scored per mouse. *, $P < 0.05$ when compared with treatment with PBS using Fisher's exact test.

PBS and dimaprit-treated mice early in infection (see Fig. 6 *A–C*). However, bacterial load in the small intestine began to diminish as the infection progressed in mice treated with dimaprit compared with mice treated with PBS (see Fig. 6 *D–F*). Taken together, these data suggest that signaling through H_2 is important for controlling the early colonization and/or growth of *Y. enterocolitica* within the PPs of mice. Additionally, these data suggest that the role of histamine, and H_2 in the immune response extends beyond allergy and is directly involved in the early cellular response to bacterial pathogens in the PPs.

Histamine signaling through H_2 is known to stimulate gastric acid secretion in the stomach. Antagonism of H_2 inhibits gastric acid secretion and, thus, results in increased stomach pH (36). Because we used the oral infection model of *Y. enterocolitica* disease and because i.p. administration of cimetidine might potentially increase the stomach pH of the mice, as a control we wanted to determine if an artificial increase in pH would alter the progression of *Y. enterocolitica* by using an alternative system. Administration of the proton pump inhibitor (PPI) omeprazole is a very potent and long lasting way in which to increase stomach pH (37). Therefore, we used oral omeprazole treatment to observe the effects of increased stomach pH on the outcome of *Y. enterocolitica*-induced disease. Treatment of mice with omeprazole before administration of *Y. enterocolitica* did not result in a difference in mouse survival or colonization of the PPs compared with untreated mice (Table 2 and Fig. 2*A*). This suggests that increased stomach pH does not alter the lethal effects caused by *Y. enterocolitica* infection nor its ability to initially invade and colonize the PPs of the small intestine.

Discussion

The development of a disease state initiated by introducing a bacterial pathogen into a susceptible host is the net product of both bacterial and host factors. Bacterial pathogens have developed complex systems to modify the response generated by the host. Many of these systems are involved in suppressing or altering the host immune system. In many bacterial infections, however, pathology and disease still occur suggesting the production of a host response in the presence of immunomodulatory bacterial systems. This immune response may differ from responses discovered out of the context of these systems (i.e., in cell culture).

To develop a better understanding of the host response mounted in response to a bacterial pathogen, we performed large-scale transcriptional profiling of infected PPs and MLNs after infection with *Y. enterocolitica*. Functional annotation of the genes identified revealed that the up-regulated genes are primarily involved in the host response to foreign stimuli. In contrast, gene clusters representing intermediate gene regulation and down-regulation comprised genes involved in a wide variety of biological processes. A large number of the implicated cytokines and chemokines were also identified in this study, demonstrating the usefulness of *in vitro* models for studying some aspects of pathogenesis. In addition, some cytokines and chemokines such as IL-11 and IL-17 were identified, and it will be interesting to see how they affect the outcome of the infection. Given the very profound PMN response in the PPs during a *Y. enterocolitica* infection, it is perhaps not surprising to see a strong early IL-17 response. In contrast, we observed no increase in IL-10 transcript at any time during infection in either the PPs or MLNs, which does not agree with some reports regarding the induction of IL-10 by LcrV (20, 26). However, this result is consistent with recent reports both for *Y. enterocolitica* and *Yersinia pestis*-infected mice that have not detected the presence of IL-10 in tissues (14, 38, 39) and is also consistent with a microarray study using the PU5–1.8 mouse macrophage-like cell line (18). Although cell culture models are immensely useful for dissecting out pathways and the molecular details, they cannot substitute for the whole animal/tissue context, and such results reinforce the need for correlation with *in vivo* models as well.

One of the most interesting and highly up-regulated host transcripts after infection was for HDC, the enzyme responsible for production of histamine. Although the finding was not pursued, the *Hdc* gene has also been found to be up-regulated by J774 macrophages infected with *Y. enterocolitica* (19). Subsequent experiments indicated that this host response (increased production of histamine in the PPs) is important for controlling the infection, specifically through H_2. This was of particular interest because acid suppression therapy using PPI or H_2 antagonists is the most effective therapy known for patients suffering from dyspepsia or gastroesophageal reflux disease (40). Annually, 20–40% of the general population has at least one episode of either disorder (41, 42). Previous experimental and epidemiological studies have suggested that increasing stomach pH by using H_2 receptor antagonists or PPI increases the susceptibility to bacterial and parasitic infection (43–45). The current theory for the increased susceptibility to infection states that increasing stomach pH removes or reduces the strength of the nonspecific defense system provided by the stomach. However, our data suggest that in the case of *Y. enterocolitica* infection, the increase in susceptibility due to H_2 antagonism is occurring in the PPs. This finding is not surprising as *Y. enterocolitica* uses a urease to protect itself from the harsh environment of the stomach (46). Not only was histamine production observed within the PPs, suggesting a localized role for it at the site of infection, but artificial stomach acid buffering with the PPI omeprazole did not appear to influence the outcome of *Y. enterocolitica* infection.

The data presented here, along with data from other laboratories which examined the production of histamine through HDC and the physiological consequences of H_2 stimulation, enable us to develop a model for the H_2-specific host response to infection (Fig. 3). In this model, *Y. enterocolitica* LPS or other factor(s) directly leads to up-regulation of the transcription of *Hdc* and consequently production of histamine in a specific set of immune cells. The ability of

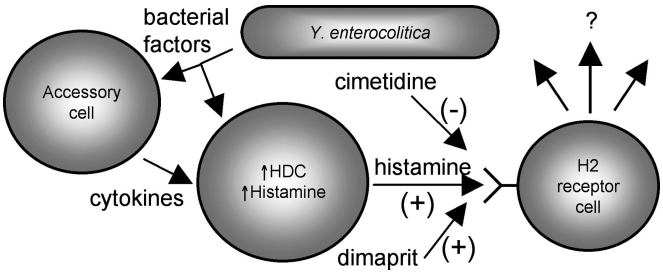

Fig. 3. Model of the histamine H_2-mediated response to *Y. enterocolitica* infection. See *Discussion* for a detailed explanation.

Y. enterocolitica to up-regulate expression of *Hdc* in J774 macrophages in culture supports this possibility (19). Alternatively, up-regulation of *Hdc* transcription and the production of histamine might be induced by cytokines produced from other cells in response to the bacterial products. *Hdc* expression has been shown to be modulated by IL-1, IL-3, IL-12, IL-18, TNF-α, granulocyte-macrophage colony-stimulating factor, and macrophage colony-stimulating factor (47–51). Another contributing factor to the observed increase in *Hdc* transcript in the PPs and MLNs and the consequent increase in histamine could be a significant influx of mast cells or basophils, the only cells known to constitutively synthesize HDC and store preformed histamine in secretory granules (29). After stimulation, the histamine, upon cellular release, would bind to cells expressing H_2. Activation of H_2 has been shown to have a variety of effects including altering the production of inflammatory cytokines and disrupting the Th1–Th2 balance during the immune response (34). The Th1–Th2 balance is known to be important for controlling *Y. enterocolitica* infection (13). However, the very early manifestations of the phenotypes described in this article suggest that the key effect of signaling of histamine through H_2 likely involves effects on early innate immune responses.

Materials and Methods

Bacterial Strains and Growth Conditions. *Y. enterocolitica* strain JB580v (serogroup O:8) was grown at 26°C for 16 h with aeration in Luria–Bertani (LB) broth supplemented with 20 μg·ml^{-1} nalidixic acid (52). Growth curves were performed at 26°C for 15 h in LB broth supplemented with different concentrations of pyrilamine, cimetidine, dimaprit, or PBS in a Bio-Tek Synergy HT plate reader (Burlington, VT). Before animal infection, bacteria were collected by centrifugation and resuspended in 1 × PBS. Colony-forming units (cfu) administered were determined by serial dilutions of the original cultures followed by plating on LB agar plates.

Animals. Female 4- to 6-week-old C57BL/6J mice were purchased from The Jackson Laboratory and allowed to acclimate for 1 week before infection. Mice were inoculated orally via a gastric tube with 0.1 ml of culture containing 5–8 × 10^9 cfu of *Y. enterocolitica*. Animal survival and tissue colonization studies were performed as described in ref. 10. PPs were identified and dissected based on visual examination of the serosal surface of the entire length of the small intestine. Luminal colonization levels were determined for three different sections of the small intestine, the proximal (8 cm), the terminal (8 cm), and the remaining middle section. The Washington University Animal Studies Committee approved all animal experiments.

GeneChip and qRT-PCR Studies. Two groups of 10 mice each were infected with 5 × 10^8 cfu of *Y. enterocolitica*. Uninfected mice and mice infected with *Y. enterocolitica* for 12 h and 2, 4, and 7 days were killed by CO_2 asphyxiation. Tissues were removed and stored in RNAlater (Ambion, Austin, TX) before total nucleic acid extraction using TRIzol reagent (Invitrogen) according to the manufacturer's instructions. Total nucleic acid was treated twice with DNase (Fisher Scientific) at 37°C to remove contaminating DNA. Extracted RNA was further purified by using the RNeasy kit (Qiagen, Valencia, CA) following the manufacturer's protocol. Purified RNA was quantified by UV absorbance at 260 and 280 nm and assessed qualitatively by formaldehyde agarose gel electrophoresis.

GeneChip hybridization and data acquisition were performed by the Siteman Cancer Center GeneChip Facility (St. Louis). All protocols were performed as recommended by the manufacturer (Affymetrix, Santa Clara, CA) and have been described elsewhere (53). The MGU74Av2 GeneChip allows for the analysis of 12,423 different transcripts. Samples obtained from two independently infected groups of mice were hybridized to separate Affymetrix MGU74Av2 GeneChips resulting in two independent data sets for each time point. The images from the scanned chips were processed by using Affymetrix GENECHIP ANALYSIS SUITE 5.0. Each image was scaled such that the average intensity value for all arrays was adjusted to 150. Duplicate infected tissue data sets were compared with two different uninfected tissue data sets resulting in a four-way comparison at each time point. The four independent comparisons made for each time point were averaged to give a single signal value used for all subsequent analysis.

Filtering methods were applied to enrich for regulated transcripts and eliminate moderate and nonregulated transcripts. To pass the filter, each transcript on the chip had to have a raw signal maximum/minimum value ratio ($Value_{MAX}/Value_{MIN}$) of >5 in the data gathered from PPs samples and 6 in the MLNs data. These values were derived empirically to enrich for genes demonstrating regulation in at least a single time point, whereas eliminating genes showing modest or no regulation between experiments. Cluster analyses (average linking) of the filtered data sets were performed by using the GENESIS (v.1.5.0) software package (54). Genes were assigned gene ontology (GO) terms (biological process level 4) using DAVID 2.0 (55). GO terms are a system of controlled vocabulary to attempt to define gene product attributes, which are maintained by the Gene Ontology Consortium (www.geneontology.org). GO terms were clustered based on the percentage of genes assigned a certain GO term from each individual cluster by using hierarchical average linking cluster analysis.

Selected genes from the GeneChip study were independently validated by qRT-PCR analysis. Three groups of five mice each were orally infected with 5 × 10^8 cfu of *Y. enterocolitica*. Tissues to be analyzed were removed from uninfected mice and mice infected for 12 h and 2, 4, and 7 days. RNA was extracted and purified as above. cDNA synthesis was performed by using Superscript III reverse transcriptase (Invitrogen). Each 25-μl qRT-PCR mixture contained 0.5 μg of cDNA, 12.5 μl of 2 × SYBR green master mixture (Qiagen), and 900 nM of gene-specific primers (see Table 5, which is published as supporting information on the PNAS web site). cDNA generated from each independent group of five mice were amplified in triplicate with a Bio-Rad iCycler. Data were normalized to GAPDH mRNA, and the relative fold-change was calculated using the $\Delta\Delta$CT method (56).

Immunohistochemistry. Immunohistochemical identification of *Y. enterocolitica* within PPs was performed using a *Y. enterocolitica* specific antibody, as described in ref. 28. Slides were scored positive for *Y. enterocolitica* if bacteria and/or bacterial colonies were readily visible under the ×40 objective. Visualization of histamine within tissue was performed as described in ref. 57. Sections of the small intestine containing PPs were removed and submerged in 4% 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide in 0.1 M sodium phosphate buffer (PB), pH 7.0, overnight and thereafter in 20% sucrose in PB. Fixed tissues were embedded in OCT compound and flash frozen. Sections (20 μm) were cut on a cryostat and fixed to glass slides. These cryosections were washed in PBS containing 0.25% Triton X-100 (PBS-T) and incubated overnight with rabbit anti-histamine antiserum diluted 1:1000 in PBS-T containing 1%

normal goat serum (58). After a PBS-T wash, sections were incubated with FITC-conjugated swine anti-rabbit IgG (Invitrogen) diluted 1:40 for 1 h. Sections were subsequently counterstained with bisbenzamide-H 33342 trihydrochloride (Sigma) and visualized with fluorescence microscopy.

Administration of Histamine Agonists, Antagonists, and a PP Inhibitor. To assess the effects of histamine via H_1 and H_2, we used the H_1 antagonist pyrilamine (5 mg·kg^{-1} body weight), the H_2 antagonist cimetidine (20 mg·kg^{-1} body weight), and the H_2 agonist dimaprit (200 mg·kg^{-1} body weight) (Tocris Cookson, Bristol, U.K.). These doses were chosen based on a previous study (59). Each was administered i.p. immediately before oral inoculation of 5–8 $\times$ 10^8 cfu of *Y. enterocolitica* and once a day thereafter until the conclusion of the experiment. The PPI omeprazole (400 μmol·kg^{-1} body weight) (Sigma) or the omeprazole diluent 0.25% (hydroxypropyl)-methyl cellulose (Sigma) were administered orally 30 min before *Y. enterocolitica* inoculation and every day thereafter until the conclusion of the experiment (37).

Statistical Analysis. All statistical analyses were performed using GraphPad PRISM v.3.03 (GraphPad, San Diego, CA). A *t* test or Fisher's exact test was used to compare groups. Survival curves were constructed using the Kaplan–Meier method and compared using the Mantel–Haenszel log-rank test (60). A *P* value of <0.05 was considered statistically significant.

Online Supporting Information Tables. Tables 3 and 4 contain signal values and gene lists obtained from *Y. enterocolitica*-infected PPs and MLNs, respectively.

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