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Wild-Type and Interleukin-10-Deficient Regulatory T Cells Reduce Effector T-Cell-Mediated Gastroduodenitis in Rag2$^{-/-}$ Mice, but Only Wild-Type Regulatory T Cells Suppress *Helicobacter pylori* Gastritis$^{\nabla}$

Chung-Wei Lee,[1,2] Varada P. Rao,[1] Arlin B. Rogers,[1] Zhongming Ge,[1] Susan E. Erdman,[1] Mark T. Whary,[1] and James G. Fox[1,2]*

Division of Comparative Medicine[1] and Biological Engineering Division,[2] Massachusetts Institute of Technology, Cambridge, Massachusetts

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CD4$^+$ CD45RB^{hi} CD25$^-$ effector T cells (T_E) promote *Helicobacter pylori* gastritis in mice, and CD4$^+$ CD45RB^{lo} CD25$^+$ regulatory T cells (T_R) are anti-inflammatory. Using adoptive transfer into *H. pylori*-infected Rag2$^{-/-}$ mice, we evaluated effects of wild-type (wt) C57BL/6 or congenic interleukin-10-deficient (IL-10$^{-/-}$) T_R cells on gastritis, gastric cytokines, and *H. pylori* colonization. Infected Rag2$^{-/-}$ mice colonized in the corpus and antrum with 10^5 to 10^6 *H. pylori* CFU/gram without associated gastritis. T_E cell transfer caused morbidity and an *H. pylori*-independent pangastritis and duodenitis (gastroduodenitis) associated with increased expression of gamma interferon (IFN-γ) and tumor necrosis factor alpha. T_E cell transfer to *H. pylori*-infected mice led to additive corpus gastritis associated with inflammatory cytokine expression and reduced colonization. wt T_R cells reduced morbidity, *H. pylori* corpus gastritis, gastroduodenitis, and inflammatory cytokine expression and reversed the decline in *H. pylori* colonization attributable to T_E cells. Although less effective than wt T_R cells, IL-10$^{-/-}$ T_R cells also reduced morbidity and gastroduodenitis but did not reduce *H. pylori* corpus gastritis or impact T_E cell inhibition of colonization. Gastric tissues from mice receiving wt T_R cells expressed higher levels of Foxp3 compared to recipients of IL-10$^{-/-}$ T_R cells, consistent with lower regulatory activity of IL-10$^{-/-}$ T_R cells. These results demonstrate that wt T_R cells suppressed T_E-cell-mediated *H. pylori*-independent gastroduodenitis and *H. pylori*-dependent corpus gastritis more effectively than IL-10$^{-/-}$ T_R cells. Compartmental differences in T_E-cell- and *H. pylori*-mediated inflammation and in regulatory effects between wt T_R and IL-10$^{-/-}$ T_R cells suggest that IL-10 expression by wt T_R cells is important to regulatory suppression of gastric inflammation.

Helicobacter pylori infects the human stomach and causes gastritis, with a subset of patients developing peptic ulcer disease, gastric carcinoma, and gastric mucosa-associated lymphoid tissue lymphoma (20, 32). Infection of C57BL/6 mice with *H. pylori* or *Helicobacter felis* results in chronic active gastritis (26, 27) and has been used to study the immune basis of *H. pylori*-induced gastritis. Helicobacter infections in humans and mice induce a Th1-predominant immune response with activation of CD4$^+$ T lymphocytes and expression of proinflammatory cytokines such as gamma interferon (IFN-γ) (24, 30). This cell-mediated immunity results in gastritis characterized by mononuclear cell infiltrates, mucosal hyperplasia, and intestinal metaplasia. In contrast, immunodeficient B6.129S7-*Rag1^{tm1Mom}* mice that lack mature B and T cells colonized at high density with *H. felis* but developed only minimal gastritis (37), indicating the importance of the adaptive immune response to helicobacter-associated gastric disease.

Recent data have demonstrated that different subpopulations of CD4$^+$ T lymphocytes play diverse roles in mediating and regulating *H. pylori*-induced gastritis. In B6.CB17-*Prkdc^{scid}* mice, adoptive transfer of wild-type (wt) CD4$^+$ CD45RB^{hi} effector T (T_E) cells from naïve donors caused severe gastritis in *H. pylori*-infected recipients, while cotransfer of wt CD4$^+$ CD45RB^{lo} regulatory T (T_R) cells protected against development of gastritis (11). T_R cells have also been defined by expression of the interleukin-2 (IL-2) receptor α chain and Foxp3 (4), the forkhead transcription factor critical to thymic selection of CD4$^+$ CD25$^+$ T_R cells (21). Depletion of CD25$^+$ Foxp3$^+$ T_R cells in *H. pylori*-infected C57BL/6 mice led to loss of immune regulation and more severe gastritis (34), as did adoptive transfer of lymphocytes depleted of CD4$^+$ CD25$^+$ cells into *H. pylori*-infected B6.Cg-*Foxn1^{nu}* (*nu*/*nu*) recipients (35). Of the many T-cell subsets with ascribed regulatory function (22), cell sorting experiments commonly use CD4$^+$ CD25$^+$ CD45RB^{lo} as naturally occurring T_R cells. However, the mechanism(s) for regulation by this type of T_R cell is not fully understood.

In adoptive transfer models using coadministration of wt T_E cells and wt or IL-10-deficient (IL-10$^{-/-}$) T_R cells, IL-10 has been shown to be essential for the function of T_R cells in suppressing inflammatory bowel disease, dysplasia, and cancer of the colon (3, 13). Based on this evidence, we evaluated *H. pylori* gastritis in B6.129S6-Rag2^{tm1Fwa} (Rag2$^{-/-}$) mice that had received wt CD4$^+$ CD25$^-$ CD45RB^{hi} T_E cells and CD4$^+$ CD25$^+$ CD45RB^{lo} T_R cells from either wt C57BL/6 or congenic IL-10$^{-/-}$ mice. The results demonstrate that T_E cells mediated a gastroduodenitis in Rag2$^{-/-}$ mice independently of *H. pylori* infection and that wt T_R cells suppressed this lesion to a greater extent than IL-10$^{-/-}$ T_R cells. Only wt T_R cells

* Corresponding author. Mailing address: Massachusetts Institute of Technology, 77 Massachusetts Ave., Cambridge, MA 02139. Phone: (617) 253-1735. Fax: (617) 258-5708. E-mail: jgfox@mit.edu.

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TABLE 1. Gastric lesions at 16 and 20 WPI with *H. pylori*

Group $(n)^a$	WPI	*H. pylori*	T_E cells	T_R cells	Median (range) of lesion index[b]										
					Corpus						Antrum				
					I	ED	H	IM	A	MM	Total	I	BGD	D	Total
wt (2/2)	20	−	−	−	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
wt (3/3)	20	+	−	−	2 (2–2.5)	1 (1–2)	1 (0.5–1)	1 (0.5–1)	2.5 (2.5)	0 (0–1)	8 (6.5–9)	0 (0)	0 (0)	0 (0)	0 (0)
Rag (7/7)	20	−	−	−	0 (0–0.5)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0–0.5)	0 (0)	0 (0)	0 (0)	0 (0)
H. pylori (11/11)	20	+	−	−	0.5 (0–1.5)	0 (0–0.5)	0 (0)	0 (0)	0 (0–1)	0 (0–1.5)	0.5 (0–2.5)	0 (0)	0 (0)	0 (0)	0 (0)
T_E (3/10)	16	−	+	−	1.5 (1.5–2)[f]	0.5 (0.5)[f]	1.5 (0.5–2)	1 (0.5–1)	3 (2–3)	2 (2)	8 (5–8)	2 (2–2.5)	4 (4)	1 (1)[f]	9 (8–9)
H. pylori+T_E (9/12)	16	+	+	−	2.5 (2–3)[d,f]	1 (0.5–2)[c,f]	1 (1–2)[d]	0.5 (0–1.5)	3 (2–3.5)	1.5 (0.5–3)[d]	8.5 (6–11.5)[e]	1.5 (1.5–2.5)[e]	3.5 (2.5–4)[c,d]	0.5 (0.5–1)[c,d,f]	7.5 (6.5–10)[d,e]
H. pylori+T_E+wt T_R (6/7)[e]	20	+	+	wt	1 (0.5–1.5)[d]	0.5 (0.5–1)[c]	1 (0.5–1)[d,g]	0.5 (0–0.5)	3 (2.5–2.5)	3 (2.5–3.5)[d]	5 (4.5–5.5)[e]	1 (0.5–2)[c]	2.5 (0.5–3)[d]	0 (0–0.5)[d]	4.5 (3–6.5)[e]
H. pylori+T_E+IL-$10^{-/-}$ T_R (4/6)[d]	20	+	+	IL-$10^{-/-}$	2 (1.5–3)	0.5 (0.5–1)	1.25 (1–2)[g]	0.25 (0–0.5)	2.75 (2–3)	2.75 (2–3)	7 (5–9)	1.25 (1–1.5)	2.25 (2–3)[c]	0 (0–0.5)[c]	5.25 (4–6)[d]

[a] Number of mice necropsied/number of mice in group. Differences represent early morbidity.
[b] I, inflammation; ED, epithelial defect; H, hyperplasia; IM, intestinal metaplasia; A, atrophy; MM, mucous metaplasia; BGD, Bruner's gland destruction; D, dysplasia.
[c] Significant influence of T_R cells at a P of < 0.05.
[d] Significant influence of T_R cells at a P of < 0.01.
[e] Significant influence of T_R cells at a P of < 0.001.
[f] Significant influence of *H. pylori* infection at a P of < 0.05.
[g] Significant difference between wt and IL-$10^{-/-}$ T_R cells at a P of < 0.05.

suppressed additive corpus gastritis attributable to *H. pylori* infection. The data support compartmental differences in T_E and *H. pylori*-mediated inflammation of the stomach and differences in regulatory effects between wt and IL-$10^{-/-}$ T_R cells, suggesting that IL-10 expression by wt T_R cells is important to regulatory suppression of gastric inflammation.

MATERIALS AND METHODS

Experimental groups. C57BL/6 wt cell donor mice and adoptive transfer recipient Rag2 gene knockout mice (B6.129S6-Rag2^{tm1Fwa} or Rag2$^{-/-}$), backcrossed 12 generations to B6 wt mice, were originally from Taconic Farms (Germantown, NY). IL-10 knockout cell donor mice (B6.129P2-*Il10*^{tm1Cgn}/J or IL-$10^{-/-}$), backcrossed 10 generations to B6 wt mice, were originally from the Jackson Laboratory (Bar Harbor, ME). Mice were bred and maintained in a facility accredited by the Association for Assessment and Accreditation of Laboratory Animal Care in static microisolator cages under specific-pathogen-free conditions as previously described (16). Male and female, 6- to 8-week old, helicobacter-free Rag2$^{-/-}$ mice were randomly assigned to uninfected or *H. pylori*-infected groups that were further subdivided 4 weeks later into groups that received either no T cells, wt T_E cells, or wt T_E cells in combination with wt or IL-$10^{-/-}$ T_R cells. Helicobacter-free C57BL/6 mice were also dosed with the same inoculum of *H. pylori* to confirm that the mouse-adapted strain induced robust gastritis in wt mice. Some mice died acutely or were euthanized at earlier time points due to declining body condition. Only data from mice surviving to the predetermined necropsy time points of 16 and 20 weeks postinfection with *H. pylori* (12 and 16 weeks postadoptive transfer, respectively) were analyzed. The protocol was approved by the Committee on Animal Care of the Massachusetts Institute of Technology.

***H. pylori* infection.** *H. pylori* Sydney strain 1 was used for oral inoculation as described previously (16, 19). *H. pylori* was grown for 24 h at 37°C under microaerobic conditions in brucella broth with 10% fetal bovine serum. The inoculum was suspended in phosphate-buffered saline to an optical density at 600 nm of 1.000 and then assessed by Gram stain and phase microscopy for purity, morphology, and motility as well as for urease, catalase, and oxidase activity. Mice were gavaged with 0.2 ml every other day for three doses. Control groups were given 0.2 ml of phosphate-buffered saline.

Cell sorting and adoptive transfer. Single-cell suspensions from spleens and mesenteric lymph nodes of wt or IL-$10^{-/-}$ mice were prepared as described previously (12). In brief, CD4$^+$ cells were isolated by using CD4 Dynabeads and CD4 DETACHaBEAD (Dynal, Oslo, Norway). Cells were then labeled with anti-CD4-Cy, anti-CD45RB-fluorescein isothiocyanate, and anti-CD25-phycoerythrin antibodies (Pharmingen, La Jolla, CA) and then sorted by flow cytometry (model Mo-flo; Cytomation Inc., Fort Collins, CO) to a purity of >95% for wt T_E cells (CD4$^+$ CD25$^-$ CD45RB^{hi}) and wt or IL-$10^{-/-}$ T_R cells (CD4$^+$ CD25$^+$ CD45RB^{lo}). Anesthetized Rag2$^{-/-}$ mice were injected in the retroorbital sinus with 3×10^5 T_E cells alone or in combination with 3×10^5 wt or IL-$10^{-/-}$ T_R cells suspended in 200 μl of Hanks balanced salt solution.

Histological evaluation. At necropsy, the stomach and proximal duodenum were removed and cut along the greater curvature. Linear gastric strips from the lesser curvature were fixed overnight in 10% neutral-buffered formalin, embedded, cut at 4 μm, and stained with hematoxylin and eosin (H-E). Lesions were scored by a veterinary pathologist blinded to sample identity, as described previously (36). Total lesion indices were calculated by the addition of individual scores for each assessment described in Table 1 except for mucous metaplasia and hyalinosis, which have been observed to develop spontaneously in mice (23) as well as from *H. felis* infection (15, 43).

Special stains and immunohistochemistry. Selective tissues were characterized using special stains and immunohistochemistry. Acidic (intestinal type) mucins were demonstrated using pH 2.5 Alcian blue followed by periodic acid-Schiff to stain remaining neutral (gastric type) mucins (36). Macrophages were stained with monoclonal antibody F4/80 (1:150; Caltag Laboratories, Burlingame, CA) and with an avidin-biotin-peroxidase complex kit (Vector Laboratories, Burlingame, CA) according to the manufacturer's instructions.

RNA extraction and quantitative PCR for cytokine expression and Foxp3. Stomach tissue was harvested and snap-frozen in liquid nitrogen. Total RNA was extracted with Trizol reagent (Invitrogen, Carlsbad, CA). cDNA was synthesized from 5 μg of total RNA using a High Capacity cDNA Archive kit (Applied Biosystems, Forster City, CA). Levels of IFN-γ, tumor necrosis factor alpha (TNF-α), IL-4, IL-10, and Foxp3 were quantified with SYBR Green PCR reagent (QIAGEN, Valencia, CA) in an ABI Prism Sequence Detection System 7700 (Applied Biosystems) per the manufacturer's instructions. Primers were

designed by Lasergene software (DNASTAR, Madison, WI). Sequences of primers were as follows: for IFN-γ, CATGGCTGTTTCTGGCTGTTACTG (forward [F]) and GTTGCTGATGGCCTGATTGTCTTT (reverse [R]) annealing at 56°C; for TNF-α, CATCTTCTCAAAATTCGAGTGACAA (F) and TGGGAG TAGACAAGGTACAACCC (R) annealing at 60°C; for IL-4, ACAGGAGAA GGGACGCCAT (F) and GAAGCCCTACAGACGAGCTCA (R) annealing at 60°C; for IL-10, GGTTGCCAAGCCTTATCGGA (F) and ACCTGCTCCACT GCCTTGCT (R) annealing at 60°C; for Foxp3, CCCAGGAAAGACAGCAA CCTT (F) and CTCACAACCAGGCCACTTGCA (R) annealing at 60°C; for glyceraldehyde-3-phosphate dehydrogenase (GAPDH),TCCATGACAACTTT GGCATTG (F) and TCACGCCACAGCTTTCCA (R) annealing at 60°C. The final concentration of each primer was 0.3 μM. Tenfold dilutions (10^7 to 10^1 copies) of each cytokine cDNA plasmid were used to generate standard curves. Expression levels, presented as cytokine copy numbers or as ratios to GAPDH (Foxp3), were normalized to the internal control (GAPDH).

Quantitative culture of *H. pylori*. Colonization of *H. pylori* in the stomach was assessed by quantitative culture as described previously (16). Briefly, tissues from corpus and antrum were weighed and homogenized in 250 μl of brucella broth using a sterile glass tissue grinder. The homogenate was serially diluted 10- and 100-fold in brucella broth and plated onto selection plates containing 5% horse blood, 250 μg/ml amphotericin B, 7 μg/ml bacitracin, 10.7 μg/ml nalidixic acid, 3.3 μg/ml polymyxin, and 100 μg/ml vancomycin. Plates were incubated microaerobically at 37°C for 7 days. Bacterial colonies were counted, and the number of CFU per gram of tissue was calculated.

Statistical analysis. Morbidity between groups was compared by the log rank test. Lesion scores were compared by a Mann-Whitney U test or by a Kruskal-Wallis one-way analysis of variance with Dunnett's test. Cytokine and Foxp3 expression levels and *H. pylori* colonization data (after log transformation) were compared using the Newman-Keuls test. Statistical analysis was performed using commercial software (Graphpad Prism 4.0; San Diego, CA) with significance at a P value of <0.05.

RESULTS

$Rag2^{-/-}$ T_E-cell-recipient mice developed morbidity and gastroduodenitis independent of *H. pylori* infection. *H. pylori*-infected and helicobacter-free control $Rag2^{-/-}$ mice that did not receive cell transfers were clinically normal throughout the 20-week study period. In contrast, 7 of 10 uninfected T_E-cell-recipient and 3 of 12 *H. pylori*-infected T_E-cell-recipient $Rag2^{-/-}$ mice died acutely or developed diarrhea and declining body condition, necessitating early euthanasia between 4 and 12 weeks posttransfer of T cells (Table 1). All remaining uninfected and *H. pylori*-infected $Rag2^{-/-}$ mice that had received T_E cells alone were necropsied at 12 weeks posttransfer of T cells, which was also 16 weeks postinfection (wpi) with *H. pylori*.

In all uninfected T_E-cell-recipient $Rag2^{-/-}$ mice, the entire alimentary tract including the stomach through the colon was grossly edematous. The stomach and proximal duodenum from all mice were evaluated histologically, and there was severe pangastritis that involved the squamous and glandular compartments of the stomach with inflammation extending into the proximal duodenum (Table 1 and Fig. 1). This T_E-cell-mediated gastroduodenitis was characterized by extensive infiltration of the mucosa and submucosa with lymphocytes, macrophages, eosinophils, and neutrophils. Other histological changes included hypertrophy and orthokeratotic hyperkeratosis of the squamous portion of the stomach, as well as epithelial defects, hyalinosis, and mucous metaplasia of the corpus, along with mild blunting, atrophy, and fusion of duodenal villi (Table 1 and Fig. 1). A consistent feature of the gastroduodenitis was loss of Brunner's glands through a combination of atrophy and dysplasia, accompanied by metaplasia to a tubuloductular phenotype (Fig. 1). The bowel distal to the proximal duodenum was examined histologically in three of these mice and had inflammatory infiltrates in the cecum and colon (data not shown), which is consistent with a previous report of T_E-cell transfer-associated colitis in $Rag2^{-/-}$ mice (8).

Adaptive immunity is required to develop *H. pylori*-associated gastritis. When evaluated at 20 wpi, the stomach and intestinal tract of *H. pylori*-infected $Rag2^{-/-}$ mice appeared grossly normal and were similar to those from uninfected $Rag2^{-/-}$ mice. Histologically, *H. pylori*-infected $Rag2^{-/-}$ mice developed only minimal corpus gastritis with scattered neutrophils (Table 1 and Fig. 2) and $F4/80^+$ macrophages in the submucosa (not shown). *H. pylori*-infected wt mice were evaluated to confirm robust gastritis from infection with *H. pylori* Sydney strain 1. These mice had moderate gross thickening of the stomach, accompanied by histological lesions consisting of significant infiltration with mononuclear cells and neutrophils, epithelial defects, oxyntic atrophy, intestinal metaplasia, and hyperplasia (Table 1) ($P < 0.001$), as previously reported (27). These findings indicated that *H. pylori*-associated gastritis in mice was promoted by adaptive immunity and that inflammation observed in *H. pylori*-infected, T_E-cell-recipient $Rag2^{-/-}$ mice was attributable to the inflammatory activity of donor T_E cells.

***H. pylori* infection exacerbated corpus gastritis in $Rag2^{-/-}$ T_E-cell-recipient mice.** *H. pylori*-infected $Rag2^{-/-}$ mice that received T_E cells at 4 wpi and were necropsied at 16 wpi were clinically affected to a similar degree as uninfected $Rag2^{-/-}$ mice that received T_E cells alone. Unexpectedly, *H. pylori*-infected, T_E-cell-recipient $Rag2^{-/-}$ mice had less mortality than uninfected T_E-cell recipients, with 9 of 12 mice surviving to the time point of 16 wpi ($P < 0.05$) (Table 1). The infected T_E-recipient mice developed similar gross changes in the stomach and intestinal tract with similar histological evidence of gastroduodenitis (Table 1 and Fig. 2). However, corpus gastritis was notably more severe in the *H. pylori*-infected T_E-recipient mice compared to the uninfected T_E recipients ($P < 0.05$) (Table 1). Otherwise, total lesion indices were similar ($P = 0.20$) (Table 1) between *H. pylori*-infected and uninfected T_E-cell-recipient $Rag2^{-/-}$ mice, suggesting that T_E cells mediated the antral gastritis, duodenitis, and destruction of Brunner's glands. These results also indicate that *H. pylori* infection further promoted the corpus gastritis that was superimposed on *H. pylori*-independent gastroduodenitis.

Adoptive transfer of wt or IL-$10^{-/-}$ T_R cells reduced morbidity and gastroduodenitis, but only wt T_R cells reduced severity of *H. pylori* corpus gastritis. Clinical morbidity posttransfer of T_E cells into *H. pylori*-infected $Rag2^{-/-}$ mice was less severe when either wt ($P < 0.001$) or IL-$10^{-/-}$ T_R cells ($P < 0.01$) were cotransferred (Table 1). Total lesion indices for T_E-cell-associated gastroduodenitis that developed independently of *H. pylori* infection were lower in $Rag2^{-/-}$ mice cotransferred with either wt T_R ($P < 0.001$) or IL-$10^{-/-}$ T_R cells ($P < 0.01$) (Table 1). wt T_R cells were more efficacious than IL-$10^{-/-}$ T_R cells in ameliorating antral gastritis ($P < 0.01$ and $P < 0.10$, respectively), Brunner's gland destruction ($P < 0.01$ and $P < 0.05$, respectively), and antral dysplasia ($P < 0.01$ and $P < 0.05$, respectively). Comparable to *H. pylori*-infected wt mice, gastritis in the *H. pylori*-infected $Rag2^{-/-}$ mice that received T_E cells and either type of T_R cells was concentrated in the corpus (Table 1 and Fig. 2), with mild

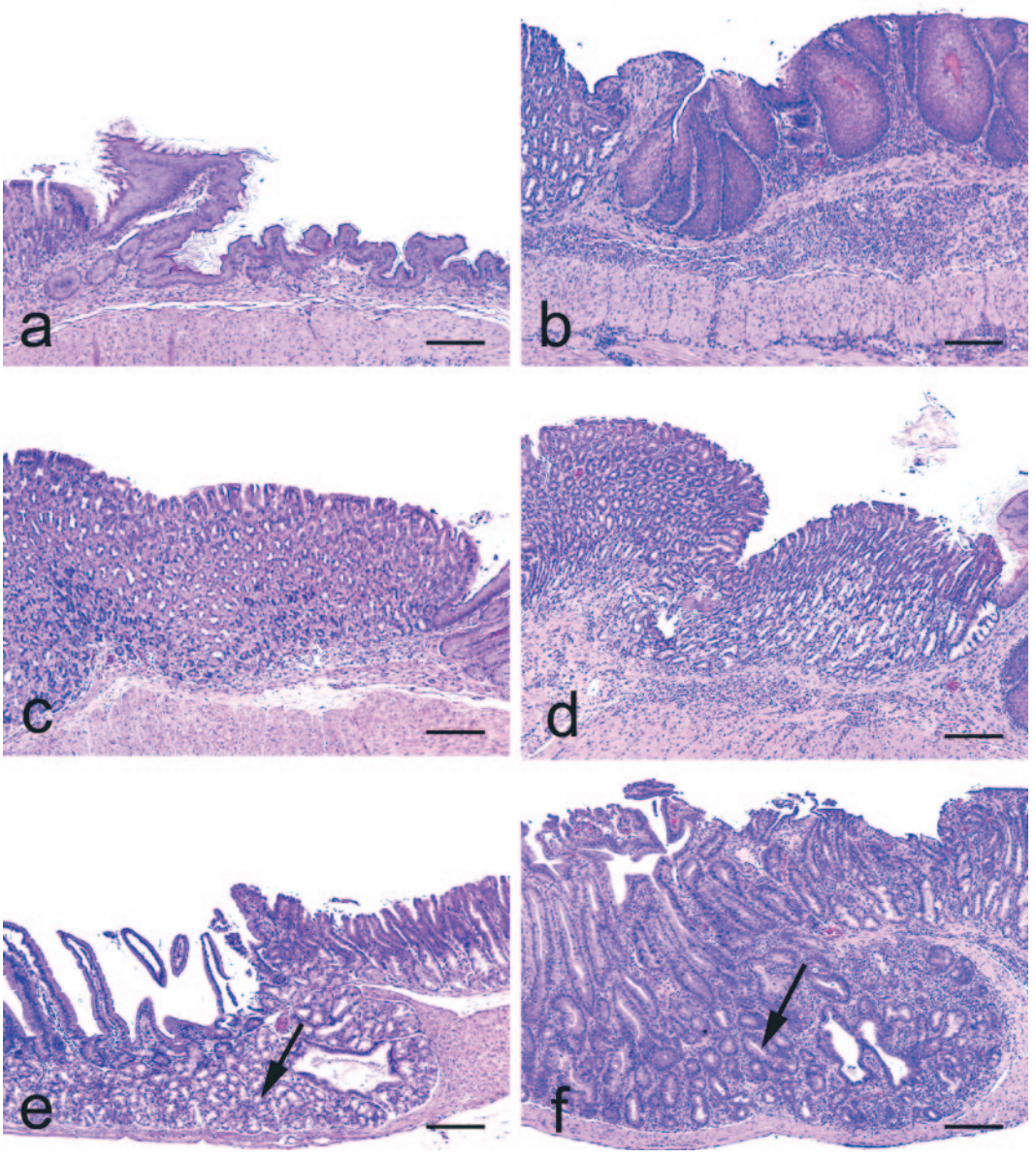

FIG. 1. Transfer of wt T_E cells into $Rag2^{-/-}$ mice resulted in immune-mediated pangastritis and gastroduodenitis. (a) Normal squamous gastric compartment of $Rag2^{-/-}$ mouse that did not receive T_E cells. (b) T_E-cell transfer resulted in inflammation of the squamous stomach with reactive epithelial cell hypertrophy and plication of the surface mucosa. (c) T_E-cell transfer also caused inflammation in the cardia and corpus. (d) T_E-cell-mediated gastritis of the glandular and oxyntic mucosa resulted in oxyntic atrophy characterized by loss of parietal and chief cells. (e) Normal Brunner's glands (arrow) in the proximal duodenum. (f) T_E-cell transfer produced duodenitis with near-complete loss of Brunner's glands due to atrophy and tubuloductular metaplasia (arrow). H-E staining was used for these samples. Bar, 200 μm.

inflammation in adjacent compartments. Notably, the T_E-cell-mediated corpus pathology in *H. pylori*-infected $Rag2^{-/-}$ mice was diminished to the greatest extent in mice that were cotransferred with wt T_R cells ($P < 0.001$), in contrast to the lack of a significant effect of IL-$10^{-/-}$ T_R cells on total lesion indices. None of the individual pathology parameters characterizing corpus gastritis were significantly different in *H. pylori*-infected T_E-cell-recipient mice given IL-$10^{-/-}$ T_R cells from infected mice that received T_E cells alone ($P = 0.26$ and higher) (Table 1 and Fig. 2). Interestingly, cotransfer of wt T_R cells resulted in significantly greater mucous metaplasia of parietal cells than the *H. pylori*-infected $Rag2^{-/-}$ mice that received T_E cells alone ($P < 0.01$) (Fig. 2).

Th1 cytokine responses were down-regulated to a greater extent by wt than IL-$10^{-/-}$ T_R cells. Consistent with the absence of gastritis, low levels of expression for IFN-γ, TNF-α, IL-4, and IL-10 were observed in gastric samples from uninfected and *H. pylori*-infected $Rag2^{-/-}$ mice that had not received T cells (Fig. 3). T_E-cell-associated gastroduodenitis was accompanied by a significant up-regulation of IFN-γ, TNF-α, IL-4, and IL-10 ($P < 0.001$), regardless of *H. pylori* infection status ($P = 0.2$ and higher), indicating that T_E cells were the

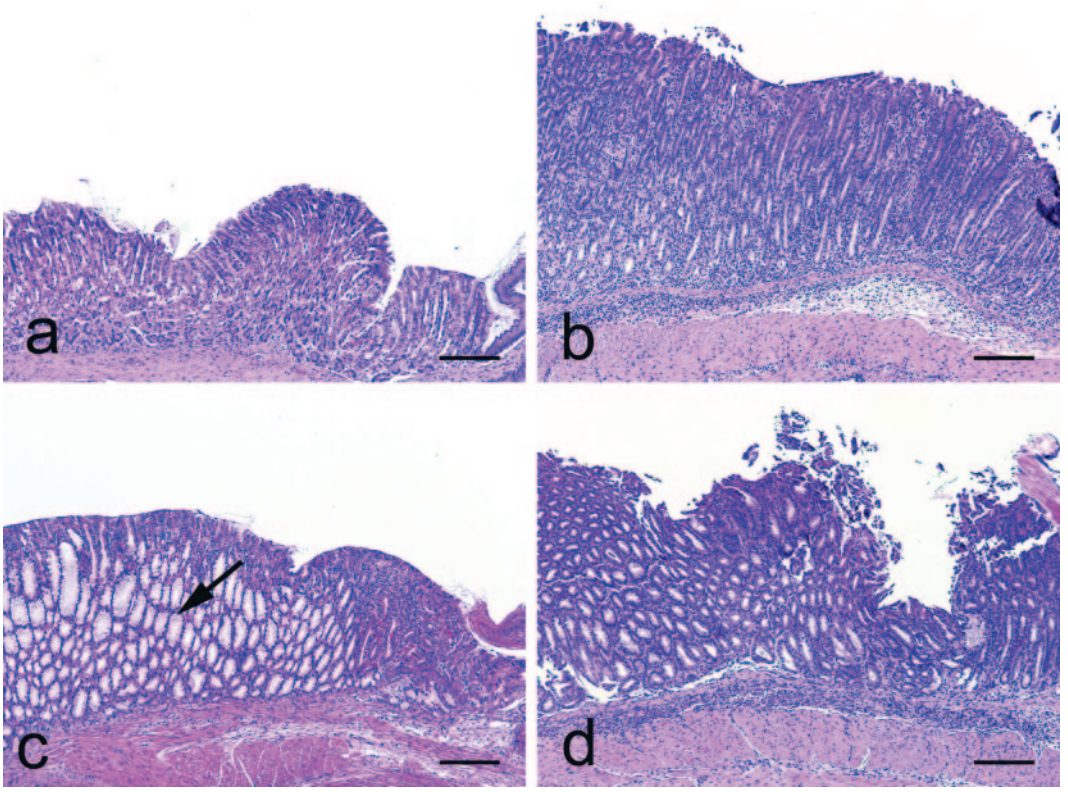

FIG. 2. *H. pylori* infection in Rag2$^{-/-}$ mice with or without subsequent transfer of wt T_E cells alone or in combination with wt or IL-10$^{-/-}$ T_R cells. (a) In the absence of T cells, *H. pylori* infection produced minimal to no gastritis. (b) *H. pylori* infection followed by transfer of T_E cells produced moderate to severe gastritis. (c) Cotransfer of wt T_R cells ameliorated severity of gastric inflammation in *H. pylori*-infected, T_E-cell-recipient mice, but for unknown reasons, marked mucous metaplasia of parietal cells developed (arrow). (d) Cotransfer of IL-10$^{-/-}$ T_R cells into *H. pylori*-infected T_E-cell-recipient mice decreased T_E-cell-mediated antral gastritis, Brunner cell loss, and epithelial dysplasia but did not reduce *H. pylori*-induced corpus gastritis. H-E staining was used for these samples. Bar, 200 μm.

main stimulus for cytokine expression. Notably, Th1-associated IFN-γ and TNF-α expression levels in gastric samples from T_E-cell-recipient, *H. pylori*-infected Rag2$^{-/-}$ mice were 2 logs higher than Th2-associated IL-4 and IL-10 mRNA levels, consistent with the proinflammatory response to gastric helicobacters (11, 14). *H. pylori*-infected Rag2$^{-/-}$ mice that received T_E plus wt T_R cells had significantly lower expression levels of IFN-γ, TNF-α, IL-4, and IL-10 ($P < 0.01$ and lower) than infected Rag2$^{-/-}$ mice that received T_E cells only. Consistent with amelioration of T_E-cell-mediated gastroduodenitis, cotransfer of IL-10$^{-/-}$ T_R cells also lowered mRNA expression for IFN-γ, TNF-α, and IL-4 ($P < 0.005$ and lower), but a decrease in IL-10 was not observed ($P = 0.51$). The ability of wt T_R cells, and not IL-10$^{-/-}$ T_R cells, to suppress corpus gastritis was consistent with higher expression levels for IFN-γ ($P < 0.05$) (Fig. 3a) in recipients of IL-10$^{-/-}$ T_R cells. Additionally, suppression of TNF-α mRNA levels was more significant in mice receiving wt than IL-10$^{-/-}$ T_R cells ($P < 0.01$ and $P < 0.05$, respectively) (Fig. 3b). Thus, Th1-predominant cytokine responses in *H. pylori*-infected T_E-cell-recipients were suppressed by both wt and IL-10$^{-/-}$ T_R cells, but suppression was greatest in recipients of wt T_R cells.

Foxp3 expression in gastric tissues was promoted to a greater extent by wt than IL-10$^{-/-}$ T_R cells. Uninfected and *H. pylori*-infected Rag2$^{-/-}$ mice that did not receive T_E cells had no detectable expression of Foxp3 in gastric tissues (Fig. 3e). Foxp3 expression levels were elevated in mice that received T_E cells ($P < 0.05$), and *H. pylori* infection did not further stimulate expression. Levels of Foxp3 were significantly higher in *H. pylori*-infected mice that received wt T_R cells ($P < 0.001$). Foxp3 expression in tissues from recipients of IL-10$^{-/-}$ T_R cells was not further elevated over levels observed for *H. pylori*-infected mice that received T_E cells alone.

***H. pylori* colonization levels were reduced by T_E cells and maintained when wt T_R cells were cotransferred.** *H. pylori* colonization levels have been reported to be inversely related to the severity of associated chronic gastritis in mice (38). Consistent with the absence of an inflammatory response, *H. pylori*-infected Rag2$^{-/-}$ mice that did not receive T cells were colonized at levels 3 to 5 logs higher in the corpus and antrum, respectively, than in infected, T_E-cell-recipient Rag2$^{-/-}$ mice (Fig. 4) ($P < 0.001$). *H. pylori*-infected mice that received T_E and wt T_R cells maintained colonization in the corpus at levels similar to *H. pylori*-infected mice that did not receive T_E cells ($P = 0.18$) (Fig. 4a). In contrast, *H. pylori*-infected mice that received T_E plus IL-10^{-}/$^{-}$ T_R cells had reduced corpus colonization similar to infected mice that received T_E cells alone ($P = 0.43$), suggesting that IL-10^{-}/$^{-}$ T_R cells failed to influence a T_E-cell-mediated inhibition of *H. pylori*. In the antrum, wt T_R cells maintained higher colonization levels than in in-

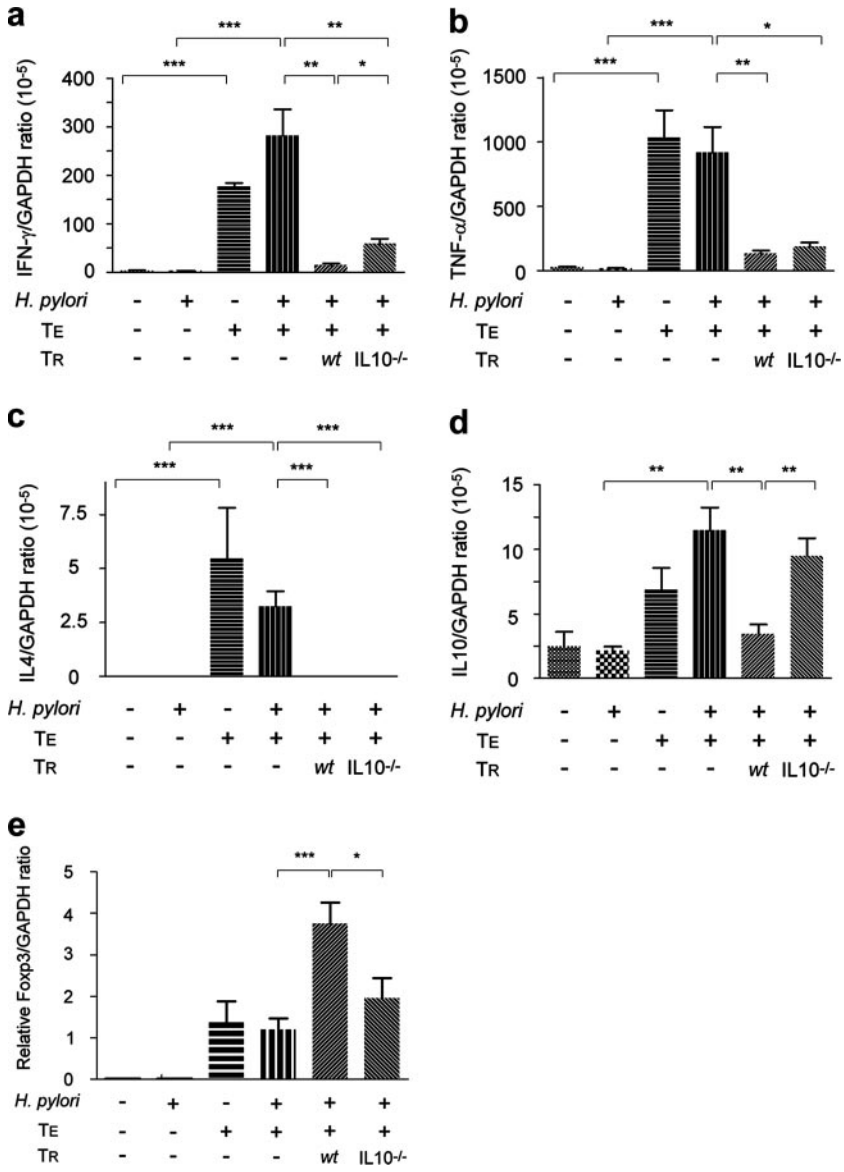

FIG. 3. Mean expression levels of mRNA for IFN-γ (a), TNF-α (b), IL-4 (c), IL-10 (d), and Foxp3 (e) were determined by quantitative PCR in gastric tissues from uninfected and *H. pylori*-infected Rag2$^{-/-}$ mice subsequently transferred with wt T_E cells alone or in combination with wt or IL-10$^{-/-}$ T_R cells. T_E cells up-regulated the expression of Th1 cytokines (IFN-γ and TNF-α) and Th2 cytokine (IL-4) with mRNA for Th1 cytokines expressed 1 to 2 logs higher than for Th2 cytokines. Cotransfer of T_E plus wt or IL-10$^{-/-}$ T_R cells suppressed expression of IFN-γ, TNF-α, and IL-4. IL-10 expression was up-regulated in T_E-cell-recipient mice while concurrent transfer of wt T_R but not IL-10$^{-/-}$ T_R cells down-regulated IL-10 expression. Foxp3 expression was significantly higher in mice that received T_E cells. Cotransfer of wt T_R cells resulted in a fourfold up-regulation of Foxp3 expression which was significantly higher than that induced by IL-10$^{-/-}$ T_R cells ($P < 0.05$), which was unchanged from mice receiving T_E cells alone. Bars represent standard error. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

fected mice that received T_E cells alone ($P < 0.05$), whereas colonization in mice receiving T_E and IL-10$^{-/-}$ T_R cells was similar to infected mice receiving T_E cells alone ($P = 0.19$) (Fig. 4b).

DISCUSSION

Using T-cell transfer into *H. pylori*-infected Rag2$^{-/-}$ mice, this study demonstrated that wt T_R cells reduced T_E cell-mediated morbidity, *H. pylori*-dependent corpus gastritis, and *H. pylori*-independent gastroduodenitis. The *H. pylori*-independent gastroduodenitis was characterized by antral gastritis, duodenitis, Brunner cell loss, and epithelial dysplasia. These lesions have not been previously described in mice with helicobacter infections and are consistent with autoimmune-like disease (22). IL-10$^{-/-}$ T_R cells also reduced T_E-cell-mediated morbidity and, to a lesser extent, *H. pylori*-independent gastroduodenitis. Notably, IL-10$^{-/-}$ T_R cells did not reduce corpus gastritis exacerbated by *H. pylori* infection, nor did IL-10$^{-/-}$ T_R cells reverse the T_E-cell-mediated reduction in *H. pylori* colonization levels. T_E cells mediated a sufficiently robust proinflammatory cytokine response that IFN-γ and

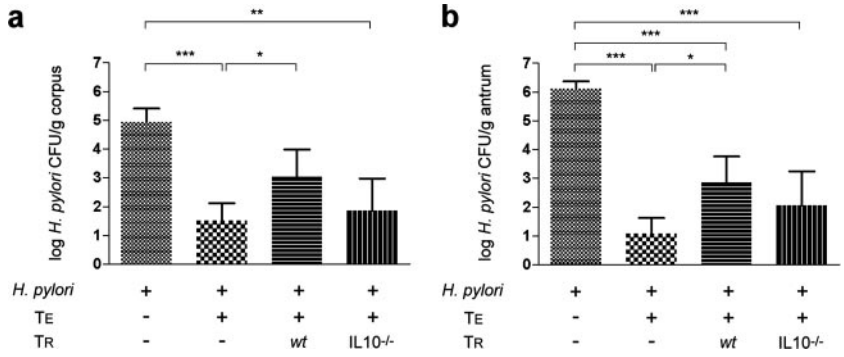

FIG. 4. Mean *H. pylori* colonization in the corpus (a) and antrum (b) of $Rag2^{-/-}$ mice receiving either no T cells, wt T_E cells, or T_E cells in combination with T_R cells from wt or IL-$10^{-/-}$ mice. *H. pylori* colonization in the corpus was lower in mice receiving T_E or T_E plus IL-$10^{-/-}$ T_R cells. Cotransfer of wt T_R cells maintained colonization levels in the corpus (only), whereas T_E plus IL-$10^{-/-}$ T_R cells significantly diminished *H. pylori* colonization in the corpus and antrum. Bars represent standard error. * $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

TNF-α expression levels were similar irrespective of *H. pylori* infection status. This Th1-predominant response was suppressed by both wt and IL-$10^{-/-}$ T_R cells, but reductions in proinflammatory cytokine levels were greatest in recipients of wt T_R cells. Gastric tissues from mice receiving wt T_R cells expressed higher levels of Foxp3 than recipients of IL-$10^{-/-}$ T_R cells, consistent with lower regulatory activity of IL-$10^{-/-}$ T_R cells. These results suggest compartmental differences in T_E-cell- and *H. pylori*-mediated gastritis and indicate that IL-10 expression by wt T_R cells is important to regulatory suppression of gastric inflammation.

Similar to *H. felis* infection in $Rag1^{-/-}$ mice (37) and despite high *H. pylori* colonization levels, $Rag2^{-/-}$ mice that did not receive T_E cells did not develop morbidity or gastrointestinal lesions. T_E-cell-recipient mice developed morbidity and gastroduodenitis independent of *H. pylori* infection, consistent with previous studies of T_E cell transfer into $Rag2^{-/-}$ mice (2, 8) and mouse models of T_R deficiency (31, 33). Observations of T_E-cell-mediated inflammation in the upper gastrointestinal tract have been reported (10, 11) but not as frequently as T_E-cell-mediated colitis (31, 33) and may be impacted by the number of cells transferred (V. P. Rao, personal communication). Extraintestinal inflammation attributable to T_E-cell infiltrates in sites such as the liver and Harderian gland have also been observed (42) and may contribute to morbidity. At these sites, T_E cells likely proliferate in response to host or, more likely, bacterial antigens either absorbed through the local epithelium or distributed systemically by vascular and lymphatic circulation. T_E-cell-mediated morbidity may also be attributable to systemic effects of up-regulation of IFN-γ, TNF-α, IL-4, IL-10, and other mediators released from gastrointestinal and extraintestinal tissues. Interestingly, preexisting *H. pylori* infection resulted in a higher survival rate in mice that subsequently received T_E cells, potentially by promoting homing of T_E cells to mucosal sites, resulting in less extraintestinal inflammation.

In uninfected T_E-cell-recipient $Rag2^{-/-}$ mice, reactive T_E cells in the gastrointestinal mucosa most likely expanded in response to dietary antigens and antigens of normal microbiota known to colonize all regions of the bowel, including the upper gastrointestinal tract of mice (39). T_E-cell-mediated pangastritis involved the squamous and glandular compartments of the stomach and extended into the duodenum. Lymphocytic infiltration of the antrum and duodenum promoted antral dysplasia, villus atrophy, and destruction of Brunner glands and was an overlapping but distinct disease pattern compared to the *H. pylori* corpus gastritis as described in this study and by others (2, 8, 41). As expected, severity of corpus gastritis was inversely correlated with *H. pylori* colonization levels, and this is indirect evidence that T_E cells were responding to *H. pylori* antigens. Both wt and IL-$10^{-/-}$ T_R cells reduced the *H. pylori*-independent gastroduodenitis, but only wt T_R cells reduced the *H. pylori* corpus gastritis and maintained higher levels of *H. pylori* colonization, suggesting compartmental or other differences in the stomach that may favor a protective role for IL-10. The anti-inflammatory effect of IL-10 is implied by the observations that IL-$10^{-/-}$ mice developed more severe *H. felis* (5) and *H. pylori* gastritis (28, 44) than congenic wt mice, as did *H. pylori*-infected B6.CB17-*Prkdc*^{scid} mice injected with IL-$10^{-/-}$ splenocytes (11). Additionally, a requirement for IL-10 competency of T_R cells has been shown in a mouse model of inflammatory bowel disease using *Helicobacter hepaticus* infection. In this model, only wt T_R cells, and notably not IL-$10^{-/-}$ T_R cells, reduced inflammation, dysplasia, and cancer lesions (13).

IL-$10^{-/-}$ T_R cells reduced morbidity and down-regulated T_E-cell-mediated gastroduodenitis along with lower IFN-γ and TNF-α expression. This observation is consistent with the absence of this distinct gastroduodenitis in uninfected or *H. pylori*-infected IL-$10^{-/-}$ mice (44) that lack IL-10-competent T_R cells. Interestingly, IL-10 expression in the $Rag2^{-/-}$ gastric tissues was not reduced in IL-$10^{-/-}$ T_R-cell-recipient mice as observed in mice that received wt T_R cells. This result suggests that IL-10 message was expressed by donor T_E or host epithelial or other inflammatory cells (6), possibly as a compensatory response of inflamed gastric tissue. Similarly, IL-10 protein levels in the *H. pylori*-infected gastric mucosa of humans have been positively associated with the severity of gastritis (6). Although the gastric IL-10 level was not sufficient to dampen *H. pylori*-mediated corpus gastritis, our findings suggest that IL-10 competency of T_R cells, but not local IL-10 per se, directly or indirectly reduces severity of *H. pylori* gastritis. Importantly, although IL-10 competence appears necessary for T_R-cell suppression of *H. pylori* gastritis, our results do not rule

out other mechanisms. For example, CTLA-4 engagement induced and maintained anergy of *H. pylori*-specific T cells in a T_R-cell-independent manner (1). Our data also show that Foxp3 expression in gastric tissues was promoted by T_E cells, suggesting differentiation of T_E cells into T_R cells in peripheral tissues as reported by others (7). Lastly, Foxp3 expression was promoted only by wt and not IL-$10^{-/-}$ T_R cells, consistent with the finding that the wt has more potent regulatory activity than IL-$10^{-/-}$ T_R cells. Further studies are necessary to clarify these issues.

In humans, *H. pylori* gastritis and progression to carcinoma have been associated with atrophy and intestinal and mucous metaplasia of fundic glands (29), consistent with the hypothesis that gastritis, gastric atrophy, and gastric cancer represent a continuum of progressive disease (18). Irrespective of *H. pylori* infection, $Rag2^{-/-}$ mice that received T_E cells developed marked mucous metaplasia in the corpus, which was more prominent in mice that received cotransfer of wt T_R cells. Fundic atrophy from chronic *H. felis* infection in mice has been associated with mucous metaplasia characterized by increased expression of trefoil factor 2 (TFF2), also known as spasmolytic polypeptide, by gastric mucous neck cells (15, 43). This lesion appears to be a replacement of parietal and chief cells with TFF2-secreting mucous cells that are similar in morphology to antral or Brunner's glands and has been suggested to be a precursor lesion of gastric cancer in mice (19) and in humans (9, 40). Alternatively, because $TFF2^{-/-}$ mice infected with *H. pylori* (17) or *H. felis* (25) developed more severe gastritis and hyperresponsiveness to IL-1β than wt mice, TFF2 may be a negative regulator of gastritis, and the promotion of mucous metaplasia by wt T_R cells may reflect a protective TFF2-associated response through an unknown mechanism.

In summary, our data demonstrate the role of T_E cells as one component of adaptive immunity that can trigger and sustain gastroduodenal inflammation in the absence of T_R cells. *H. pylori* infection coupled with T_E-cell transfer resulted in a more severe corpus gastritis that was compartmentally distinct from T_E-cell-mediated gastroduodenitis. Further, our results demonstrate that IL-10 competence of T_R cells appeared to be a requirement for suppression of *H. pylori*-induced gastritis but was not as critical for amelioration of T_E-cell-mediated gastroduodenitis or protection against associated morbidity. These results are compatible with previous reports that *H. pylori*-induced gastritis in mice is regulated by an IL-10-dependent, Th1-type immune response (5, 44), likely via the function of $CD25^+$ $Foxp3^+$ T_R cells (34), and is consistent with increased Foxp3 expression in wt T_R-cell recipients. Compartmental differences in T_E-cell- and *H. pylori*-mediated inflammation and differential regulation by wt or IL-$10^{-/-}$ T_R cells should prove valuable for study of bacterial and immune-mediated gastritis.

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REFERENCES

1. **Anderson, K. M., S. J. Czinn, R. W. Redline, and T. G. Blanchard.** 2006. Induction of CTLA-4-mediated anergy contributes to persistent colonization in the murine model of gastric *Helicobacter pylori* infection. J. Immunol. **176:**5306–5313.
2. **Annacker, O., R. Pimenta-Araujo, O. Burlen-Defranoux, T. C. Barbosa, A. Cumano, and A. Bandeira.** 2001. $CD25^+$ $CD4^+$ T cells regulate the expansion of peripheral CD4 T cells through the production of IL-10. J. Immunol. **166:**3008–3018.
3. **Asseman, C., S. Mauze, M. W. Leach, R. L. Coffman, and F. Powrie.** 1999. An essential role for interleukin 10 in the function of regulatory T cells that inhibit intestinal inflammation. J. Exp. Med. **190:**995–1004.
4. **Banham, A. H., F. M. Powrie, and E. Suri-Payer.** 2006. $FOXP3^+$ regulatory T cells: current controversies and future perspectives. Eur. J. Immunol. **36:**2832–2836.
5. **Berg, D. J., N. A. Lynch, R. G. Lynch, and D. M. Lauricella.** 1998. Rapid development of severe hyperplastic gastritis with gastric epithelial dedifferentiation in *Helicobacter felis*-infected IL-$10^{-/-}$ mice. Am. J. Pathol. **152:** 1377–1386.
6. **Bodger, K., J. I. Wyatt, and R. V. Heatley.** 1997. Gastric mucosal secretion of interleukin-10: relations to histopathology, *Helicobacter pylori* status, and tumour necrosis factor-alpha secretion. Gut **40:**739–744.
7. **Chen, W., W. Jin, N. Hardegen, K. J. Lei, L. Li, N. Marinos, G. McGrady, and S. M. Wahl.** 2003. Conversion of peripheral $CD4^+$ $CD25^-$ naive T cells to $CD4^+$ $CD25^+$ regulatory T cells by TGF-beta induction of transcription factor Foxp3. J. Exp. Med. **198:**1875–1886.
8. **Corazza, N., S. Eichenberger, H. P. Eugster, and C. Mueller.** 1999. Nonlymphocyte-derived tumor necrosis factor is required for induction of colitis in recombination activating gene $RAG2^{-/-}$ mice upon transfer of $CD4^+$ $CD45RB^{hi}$ T cells. J. Exp. Med. **190:**1479–1492.
9. **Dhar, D. K., T. C. Wang, R. Maruyama, J. Udagawa, H. Kubota, T. Fuji, M. Tachibana, T. Ono, H. Otani, and N. Nagasue.** 2003. Expression of cytoplasmic TFF2 is a marker of tumor metastasis and negative prognostic factor in gastric cancer. Lab. Investig. **83:**1343–1352.
10. **Dohi, T., K. Fujihashi, T. Koga, Y. Etani, N. Yoshino, Y. I. Kawamura, and J. R. McGhee.** 2004. $CD4^+$ $CD45RB^{Hi}$ interleukin-4 defective T cells elicit antral gastritis and duodenitis. Am. J. Pathol. **165:**1257–1268.
11. **Eaton, K. A., M. Mefford, and T. Thevenot.** 2001. The role of T cell subsets and cytokines in the pathogenesis of *Helicobacter pylori* gastritis in mice. J. Immunol. **166:**7456–7461.
12. **Erdman, S. E., T. Poutahidis, M. Tomczak, A. B. Rogers, K. Cormier, B. Plank, B. H. Horwitz, and J. G. Fox.** 2003. $CD4^+$ $CD25^+$ regulatory T lymphocytes inhibit microbially induced colon cancer in Rag2-deficient mice. Am. J. Pathol. **162:**691–702.
13. **Erdman, S. E., V. P. Rao, T. Poutahidis, M. M. Ihrig, Z. Ge, Y. Feng, M. Tomczak, A. B. Rogers, B. H. Horwitz, and J. G. Fox.** 2003. $CD4^+$ $CD25^+$ regulatory lymphocytes require interleukin 10 to interrupt colon carcinogenesis in mice. Cancer Res. **63:**6042–6050.
14. **Fox, J. G., P. Beck, C. A. Dangler, M. T. Whary, T. C. Wang, H. N. Shi, and C. Nagler-Anderson.** 2000. Concurrent enteric helminth infection modulates inflammation and gastric immune responses and reduces helicobacter-induced gastric atrophy. Nat. Med. **6:**536–542.
15. **Fox, J. G., X. Li, R. J. Cahill, K. Andrutis, A. K. Rustgi, R. Odze, and T. C. Wang.** 1996. Hypertrophic gastropathy in *Helicobacter felis*-infected wild-type C57BL/6 mice and p53 hemizygous transgenic mice. Gastroenterology **110:**155–166.
16. **Fox, J. G., A. B. Rogers, M. Ihrig, N. S. Taylor, M. T. Whary, G. Dockray, A. Varro, and T. C. Wang.** 2003. *Helicobacter pylori*-associated gastric cancer in INS-GAS mice is gender specific. Cancer Res. **63:**942–950.
17. **Fox, J. G., A. B. Rogers, E. A. Kurt-Jones, and T. C. Wang.** 2006. *H. pylori* accelerates the progression of dysplasia of the pylori antrum in $Tff2^{-/-}$ C57BL/6 × Sv129 infected mice. Gastroenterology **130:**A9.
18. **Fox, J. G., and T. C. Wang.** 2007. Inflammation, atrophy, and gastric cancer. J. Clin. Investig. **117:**60–69.
19. **Fox, J. G., T. C. Wang, A. B. Rogers, T. Poutahidis, Z. Ge, N. Taylor, C. A. Dangler, D. A. Israel, U. Krishna, K. Gaus, and R. M. Peek, Jr.** 2003. Host and microbial constituents influence *Helicobacter pylori*-induced cancer in a murine model of hypergastrinemia. Gastroenterology **124:**1879–1890.
20. **Hansson, L. E., O. Nyren, A. W. Hsing, R. Bergstrom, S. Josefsson, W. H. Chow, J. F. Fraumeni, Jr., and H. O. Adami.** 1996. The risk of stomach cancer in patients with gastric or duodenal ulcer disease. N. Engl. J. Med. **335:**242–249.
21. **Hori, S., T. Nomura, and S. Sakaguchi.** 2003. Control of regulatory T cell development by the transcription factor Foxp3. Science **299:**1057–1061.
22. **Jiang, S., R. I. Lechler, X. S. He, and J. F. Huang.** 2006. Regulatory T cells and transplantation tolerance. Hum. Immunol. **67:**765–776.
23. **Kang, W., S. Rathinavelu, L. C. Samuelson, and J. L. Merchant.** 2005. Interferon gamma induction of gastric mucous neck cell hypertrophy. Lab. Investig. **85:**702–715.
24. **Karttunen, R.** 1991. Blood lymphocyte proliferation, cytokine secretion and appearance of T cells with activation surface markers in cultures with

Helicobacter pylori. Comparison of the responses of subjects with and without antibodies to *H. pylori*. Clin. Exp. Immunol. **83:**396–400.
25. **Kurt-Jones, E. A., L. Cao, F. Sandor, A. B. Rogers, M. T. Whary, P. R. Nambiar, A. Cerny, G. Bowen, J. Yan, S. Takaishi, A. L. Chi, G. Reed, J. Houghton, J. G. Fox, and T. C. Wang.** 2007. Trefoil family factor 2 is expressed in murine gastric and immune cells and controls both gastrointestinal inflammation and systemic immune responses. Infect. Immun. **75:**471–480.
26. **Lee, A., J. G. Fox, G. Otto, and J. Murphy.** 1990. A small animal model of human *Helicobacter pylori* active chronic gastritis. Gastroenterology **99:**1315–1323.
27. **Lee, A., J. O'Rourke, M. C. De Ungria, B. Robertson, G. Daskalopoulos, and M. F. Dixon.** 1997. A standardized mouse model of *Helicobacter pylori* infection: introducing the Sydney strain. Gastroenterology **112:**1386–1397.
28. **Matsumoto, Y., T. G. Blanchard, M. L. Drakes, M. Basu, R. W. Redline, A. D. Levine, and S. J. Czinn.** 2005. Eradication of *Helicobacter pylori* and resolution of gastritis in the gastric mucosa of IL-10-deficient mice. Helicobacter **10:**407–415.
29. **Mera, R., E. T. Fontham, L. E. Bravo, J. C. Bravo, M. B. Piazuelo, M. C. Camargo, and P. Correa.** 2005. Long-term follow up of patients treated for *Helicobacter pylori* infection. Gut **54:**1536–1540.
30. **Mohammadi, M., S. Czinn, R. Redline, and J. Nedrud.** 1996. Helicobacter-specific cell-mediated immune responses display a predominant Th1 phenotype and promote a delayed-type hypersensitivity response in the stomachs of mice. J. Immunol. **156:**4729–4738.
31. **Morrissey, P. J., K. Charrier, S. Braddy, D. Liggitt, and J. D. Watson.** 1993. $CD4^+$ T cells that express high levels of CD45RB induce wasting disease when transferred into congenic severe combined immunodeficient mice. Disease development is prevented by cotransfer of purified $CD4^+$ T cells. J. Exp. Med. **178:**237–244.
32. **Pisani, P., D. M. Parkin, F. Bray, and J. Ferlay.** 1999. Estimates of the worldwide mortality from 25 cancers in 1990. Int. J. Cancer **83:**18–29.
33. **Powrie, F., M. W. Leach, S. Mauze, L. B. Caddle, and R. L. Coffman.** 1993. Phenotypically distinct subsets of $CD4^+$ T cells induce or protect from chronic intestinal inflammation in C. B-17 SCID mice. Int. Immunol. **5:**1461–1471.
34. **Rad, R., L. Brenner, S. Bauer, S. Schwendy, L. Layland, C. P. da Costa, W. Reindl, A. Dossumbekova, M. Friedrich, D. Saur, H. Wagner, R. M. Schmid, and C. Prinz.** 2006. $CD25^+$/$Foxp3^+$ T cells regulate gastric inflammation and *Helicobacter pylori* colonization *in vivo*. Gastroenterology **131:**525–537.
35. **Raghavan, S., M. Fredriksson, A. M. Svennerholm, J. Holmgren, and E. Suri-Payer.** 2003. Absence of $CD4^+CD25^+$ regulatory T cells is associated with a loss of regulation leading to increased pathology in *Helicobacter pylori*-infected mice. Clin. Exp. Immunol. **132:**393–400.
36. **Rogers, A. B., N. S. Taylor, M. T. Whary, E. D. Stefanich, T. C. Wang, and J. G. Fox.** 2005. *Helicobacter pylori* but not high salt induces gastric intraepithelial neoplasia in B6129 mice. Cancer Res. **65:**10709–10715.
37. **Roth, K. A., S. B. Kapadia, S. M. Martin, and R. G. Lorenz.** 1999. Cellular immune responses are essential for the development of *Helicobacter felis*-associated gastric pathology. J. Immunol. **163:**1490–1497.
38. **Sakagami, T., M. Dixon, J. O'Rourke, R. Howlett, F. Alderuccio, J. Vella, T. Shimoyama, and A. Lee.** 1996. Atrophic gastric changes in both *Helicobacter felis* and *Helicobacter pylori* infected mice are host dependent and separate from antral gastritis. Gut **39:**639–648.
39. **Sarma-Rupavtarm, R. B., Z. Ge, D. B. Schauer, J. G. Fox, and M. F. Polz.** 2004. Spatial distribution and stability of the eight microbial species of the altered Schaedler flora in the mouse gastrointestinal tract. Appl. Environ. Microbiol. **70:**2791–2800.
40. **Schmidt, P. H., J. R. Lee, V. Joshi, R. J. Playford, R. Poulsom, N. A. Wright, and J. R. Goldenring.** 1999. Identification of a metaplastic cell lineage associated with human gastric adenocarcinoma. Lab. Investig. **79:**639–646.
41. **Suri-Payer, E., and H. Cantor.** 2001. Differential cytokine requirements for regulation of autoimmune gastritis and colitis by $CD4^+$ $CD25^+$ T cells. J. Autoimmun. **16:**115–123.
42. **Theve, E., J. Fox, and A. Rogers.** 2005. Multiple autoimmune syndrome in 129/SvEv $Rag2^{-/-}$ mice receiving adoptive transfer of T effector cells. Vet. Pathol. **42:**707.
43. **Wang, T. C., J. R. Goldenring, C. Dangler, S. Ito, A. Mueller, W. K. Jeon, T. J. Koh, and J. G. Fox.** 1998. Mice lacking secretory phospholipase A2 show altered apoptosis and differentiation with *Helicobacter felis* infection. Gastroenterology **114:**675–689.
44. **Whary, M., B. Sheppard, J. Cline, S. Xu, and J. Fox.** 2001. IL-10 deficiency reduces *Helicobacter pylori* gastric colonization in C57BL/6 mice. Gastroenterology **120:**A99–A100.

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