Modulation of the CD4+ T-Cell Response by Helicobacter pylori Depends on Known Virulence Factors and Bacterial Cholesterol and Cholesterol α-Glucoside Content

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Helicobacter pylori blocks the proliferation of human CD4+ T cells, facilitated by vacuolating exotoxin (VacA) and γ-glutamyl transpeptidase (GGT). H. pylori–triggered T-cell reactions in mice correlate with bacterial cholesterol and cholesterol α-glucoside content but their role in human cells is unclear. We characterized the effect of VacA, GGT, and cholesterol on T-helper 1, T-helper 2, T-regulatory and T-helper 17 associated cytokines and T-cell proliferation. VacA, GGT, and bacterial cholesterol content exhibited differential and synergistic inhibitory effects on the expression of activation markers CD25 and CD69 and on interleukin 2, interleukin 4, interleukin 10, and interferon γ production. These factors did not affect the H. pylori–mediated abrogation of transforming growth factor β secretion or increased interleukin 6 production. Cholesterol α-glucosyltransferase–deficient bacteria exerted strongly reduced antiproliferative effects on primary human CD4+ T cells. In conclusion, H. pylori shapes rather than suppresses human CD4+ T-cell responses, and glucosylated cholesterol is a relevant bacterial component involved in this modulation.

Helicobacter pylori resides in one-half of the world’s population and can cause gastritis, ulcer, and gastric cancer [1]. T-cell responses against H. pylori are associated with gastric inflammation [2, 3] and, in animal models, confer protection against infection [4, 5]. H. pylori virulence factors have evolved to suppress protective T-cell responses. The constitutively expressed enzyme γ-glutamyl transpeptidase (GGT) is responsible for suppression of T cells [6]; however, vacuolating exotoxin (VacA) has also been implicated in the inhibition of T-cell proliferation [7]. H. pylori has been shown to inhibit interleukin 2 (IL-2) secretion from human T-cell lines in a VacA-dependent manner, with modest effects in primary human T cells [8]. A third mechanism used by H. pylori has also been proposed: H. pylori is a cholesterol auxotroph and therefore extracts this essential nutrient from epithelial and antigen-presenting cells [9]. In a murine model of H. pylori infection, this process affected the immune response against the bacteria by promoting phagocytosis by antigen-presenting cells and thereby enhancing antigen-specific T-cell responses [9].

T cells differentiate into diverse subsets upon activation by antigen-presenting cells [10], including T-helper 1 (Th1), T-helper 2 (Th2), T-helper 17 (Th17), and T-regulatory (Treg) cells [11]. Th1 cells characteristically produce interferon γ (IFN-γ) and are important for immunity against intracellular microorganisms, whereas Th2 cells produce interleukin 4 (IL-4), interleukin 5 (IL-5), interleukin 10 (IL-10), and interleukin 13 (IL-13) and are linked to immune responses against extracellular pathogens. Cellular immune responses against H.
*Helicobacter pylori* are known to be predominantly mediated by Th1 cells [2–5]. Th17 cells depend on transforming growth factor β (TGF-β) and interleukin 6 (IL-6) for differentiation, produce interleukin 17A (IL-17A), and are important in the response to extracellular bacteria at mucosal surfaces, including *H. pylori* infection [12, 13]. Treg cells, which also require TGF-β for differentiation, are characterized by the expression of the transcription factor FoxP3 and their capacity to suppress immune responses [14] and are implicated in the persistence of *H. pylori* [15].

Previous investigations into the effects of *H. pylori* on CD4+ T cells have mainly used cell lines, even though these results may not be fully applicable to primary human T cells [8]. In addition, whereas the effect of purified molecules (eg, VacA and GGT) has been tested on T cells, little is understood about their contribution to the modulation of immunity by whole bacteria [7, 8]. Moreover, because most studies have focused on IL-2 secretion [6–8], the effect of *H. pylori* on other cytokines remains poorly characterized. Finally, potential interactions between *H. pylori* virulence factors affecting T-cell immunity have not yet been addressed.

In this study, we evaluate the interdependence of the effects of VacA, GGT, and cholesterol/cholesterol α-glucosides on primary T cells using live *H. pylori* and relevant mutants. Analyzing Th1-, Th2-, and Th17-associated cytokines and Treg subsets of human CD4+ T cells and proliferation, we demonstrate that *H. pylori* shapes rather than simply suppresses immune responses and bacterial cholesterol content, and the ability to form cholesterol α-glucosides contribute to this process.

**MATERIALS AND METHODS**

**Bacteria**

*CagPAI+ H. pylori* wild-type strain P12 is a clinical isolate, which has been described elsewhere [16]. *H. pylori* P12 wild type was cultured on horse serum agar plates supplemented with vancomycin (10 μg/mL). The isogenic P12 mutant strains *H. pylori ΔvacA*, *H. pylori Δggt*, and *H. pylori Δ421* (lacking cholesterol α-glucosyl transferase) strains were cultivated on selective agar plates. Bacteria were incubated for 2 days before infection at 37°C in an incubator with 5% CO₂ and 5% O₂.

**Isolation of Primary Human CD4+ T Cells**

Peripheral blood mononuclear cells were obtained following approval of the ethical committee of the Charité and informed consent from healthy human donors as described elsewhere [17]. CD4+ T cells were isolated with a CD4+ T Cell Isolation Kit II (Miltenyi Biotech) according to the manufacturer’s instructions. CD4+ T cells were cultured in Roswell Park Memorial Institute 1640 medium (Gibco) supplemented with 10% fetal calf serum (FCS) or in Optimizer T-cell Expander serum-free medium (Gibco). The purified cells were 99% CD4+ cells, as assessed by flow cytometry.

**Incubation of CD4+ T Cells With *H. pylori***

CD4+ T cells (1 × 10⁶ cells/mL) were incubated with *H. pylori* at different multiplicities of infection (MOIs) for 2 hours. *H. pylori* or *H. pylori Δ421* were coated with water-soluble cholesterol (C4951; Sigma) at a concentration of 100 μmol/L for 30 minutes at 37°C and washed prior to infection for 2 hours. Penicillin (100 U/mL), streptomycin (100 μg/mL), and gentamycin (100 μg/mL) were then added to kill extracellular bacteria. After 1 hour, the CD4+ T cells were activated with immobilized anti-CD3 (1 μg/mL) and soluble anti-CD28 (3 μg/mL; BD Pharmingen) and incubated for 24 hours or 7 days.

**CD4+ T-Cell Proliferation Assay**

CD4+ T cells were suspended in phosphate-buffered saline (PBS) containing carboxyfluorescein succinimidyl ester (CFSE; Molecular Probes, Invitrogen) at a concentration of 0.5 mmol/L, and incubated for 3 minutes at room temperature, and washed twice with the appropriate culture medium. CFSE-stained CD4+ T cells (1 × 10⁶ cells/mL) were then suspended in Optimizer T-cell Expander serum-free medium (Gibco) and incubated with *H. pylori* or mutants, as indicated above. Cell proliferation was evaluated after 7 days by CFSE fluorescence. Dead cells were excluded from the analysis.

**Measurement of Cytokine Levels**

Secretion levels of IL-2, IL-10, and tumor necrosis factor α (TNF-α) were evaluated by the DuoSet enzyme-linked immunosorbent assay (ELISA) development system (R&D Systems). For measurement of IFN-γ, IL-6, IL-4, and IL-17A levels, sandwich ELISAs were performed as suggested by the supplier (U-Cytech, the Netherlands). The TGF-β1 ELISA was purchased from Bender MedSystems.

**Flow Cytometry and Apoptosis Assay**

CD4+ T cells were washed with PBS and 2% FCS and incubated with anti-CD25 (Bekton Dickinson) or anti-CD69 (Dako) for 30 minutes at 4°C. After washing the cells with PBS and 2% FCS, fixation was performed with 4% paraformaldehyde for 20 minutes at room temperature. For each experimental condition, data from 10 000 cells were collected on a FACS-Calibur flow cytometer (BD Biosciences). Live cells were gated based on forward and side-scatter properties. Apoptosis was evaluated with the Annexin V-FITC Apoptosis Detection Kit I from BD Pharmingen.

**Statistical Analysis**

Data shown are median values unless otherwise stated. Non-parametric tests (Mann-Whitney test or Wilcoxon paired test) or analysis of variance were used to determine statistical significance. Differences were considered significant if *P* < .05.
RESULTS

Interdependent and Differential Inhibition of Early T-Cell Activation Markers IL-2, CD25, and CD69 by H. pylori Virulence Factors

We mimicked the ability of H. pylori to obtain cholesterol from the membranes of host cells [9] by incubating the bacteria with cholesterol. The effect of cholesterol saturation in combination with VacA or GGT on IL-2 production and expression of its receptor CD25 in human CD4+ T cells was assessed by ELISA and fluorescence-activated cell sorting (FACS), respectively. Cells obtained from healthy volunteers were incubated with H. pylori and then activated. Levels of IL-2 secretion after 24 hours by CD4+ T cells from different donors not exposed to H. pylori ranged from 450 to 1300 pg/mL (Figure 1). In comparison, levels of IL-2 production were significantly reduced upon incubation with H. pylori (Figure 1A); however, coating H. pylori with cholesterol significantly attenuated this inhibition (Figure 1A). Isogenic mutants lacking VacA elicited a similar, albeit weaker, phenotype as the cholesterol-saturated H. pylori. In agreement with previous studies [6], mutants lacking GGT activity inhibited IL-2 production (Figure 1A).

We also measured the expression of CD25, a component of the IL-2 receptor, upon exposure to cholesterol-saturated H. pylori or VacA or GGT mutants. FACS analysis revealed that, similar to IL-2 production, expression of CD25 after 24 hours was significantly reduced when T cells were incubated with wild-type H. pylori, but not cholesterol-coated H. pylori or the VacA or GGT mutant bacteria (Figure 1B, upper panels). After 7 days of incubation, CD25 remained down-regulated in viable H. pylori–exposed T cells. This long-term effect was partially reverted by precoating the bacteria with cholesterol (Figure 1B, lower panel). Consistent with the data from 24-hour incubation, expression of CD25 was also not reduced in cells incubated with VacA or GGT mutant bacteria for 7 days (Figure 1B, lower panel). H. pylori also hampered the up-regulation of CD69 (Figure 1C), another early activation marker transiently expressed upon stimulation of T cells [18]. Expression of CD69 was exclusively dependent on VacA, as its inhibition was reverted by neither cholesterol coating nor the absence of GGT (Figure 1C).

These effects were not due to differences in the ability of cholesterol-treated bacteria or mutants to interact with CD4+ T cells or induce their apoptosis (Figure 2; Table 1). Therefore, these results reveal that even though H. pylori inhibits IL-2 and/or IL-2 receptor expression via the action of VacA and GGT, their effect is dramatically modulated by bacterial cholesterol content. In contrast, the inhibition of CD69 is exclusively mediated by VacA.

Interdependent Effects of H. pylori Virulence Factors on Th1- and Th2-Type Cytokine Release in Activated Human T Cells

CD4+ T cells play a key role in the immune response against H. pylori [19], exerting their protective effect mainly through the secretion of cytokines. Hence, CD4+ T cells were incubated with wild-type H. pylori or VacA and GGT mutants for 2 hours and activated. Levels of IL-10, IL-4, IFN-γ, and TNF-α were measured 24 hours later.

Cells from healthy donors released 2–350 pg/mL of IL-10. Cells from 2 donors did not produce detectable levels of the cytokine. Production of IL-10 was suppressed by H. pylori (Figure 3); however, this was restored by coating the bacteria with cholesterol or in the absence of VacA and GGT (Figure 3A). Coating of H. pylori ΔvacA or H. pylori Δggt with cholesterol did not further alter the release of IL-10 (data not shown).

Gastric epithelial cells from healthy individuals have been shown to produce more IL-4 than cells from H. pylori–infected patients [20]. In accordance, levels of IL-4 released by stimulated CD4+ T cells (2–33 pg/mL) were significantly suppressed in the presence of H. pylori. This suppression was reverted when CD4+ T cells were incubated with cholesterol-coated H. pylori (Figure 3B). VacA seemed to have the most significant impact on the inhibition of IL-4 (Figure 3B), and accordingly, coating of H. pylori ΔvacA with cholesterol had no additional effect (data not shown). By contrast, the absence of GGT did not increase IL-4 levels (Figure 3B). These data show that H. pylori suppresses IL-4 production by CD4+ T cells mainly in a VacA-dependent manner. Furthermore, the amplitude of this suppression is modulated by bacterial cholesterol content.

Mucosal inflammation in H. pylori infection correlates with Th1 T-cell signatures [21], and IFN-γ–producing T cells are involved in gastric epithelial cell cytotoxicity [22]. Levels of IFN-γ secretion by CD4+ T cells varied between 200 and 4500 pg/mL (Figure 3C). Cells from 1 donor did not release IFN-γ upon stimulation. CD4+ T cells incubated with H. pylori released less IFN-γ than cells not exposed to the bacteria (Figure 3C). Suppression of IFN-γ and IL-4, however, was less pronounced than that observed for IL-10 (Figure 3A, B, and C). Interestingly, although the presence of VacA and GGT was required for the suppression of IFN-γ, bacterial cholesterol content did not play any role.

Production of TNF-α has been shown to correlate with gastritis and bacterial load in H. pylori–infected patients [23, 24]. In contrast to the effect of H. pylori on the other cytokines studied here, exposure of CD4+ T cells to the bacterium did not modulate the secretion of this pro-inflammatory cytokine. Under all conditions, cells released TNF-α levels of 395–2, 820 pg/mL (Figure 3).

Our results show that H. pylori can suppress anti-inflammatory and pro-inflammatory cytokine production. However, modulation of the latter type is often less pronounced or may not occur. Bacterial cholesterol content affects the amplitude of the inhibition of anti-inflammatory cytokines only, whereas VacA and GGT are involved in the inhibition of both types of cytokines.

H. pylori Shapes T-Cell Response • JID 2011:204 (1 November) • 1341
Figure 1. Interdependent modulation of early T-cell activation markers interleukin 2 (IL-2), CD25, and CD69 by *Helicobacter pylori* virulence factors. Human CD4^{+} T-cells were incubated with *H. pylori*, cholesterol-coated *H. pylori* (*H. pylori* CHOL), mutant *H. pylori* lacking γ-glutamyl transpeptidase (*H. pylori*D_{ggt}), or mutant *H. pylori* lacking vacuolating exotoxin (*H. pylori*D_{vacA}) at a multiplicity of infection (MOI) of 50 for 2 hours and then activated with anti-CD3 and anti-CD28. A, IL-2 levels. Supernatants were harvested after 24 hours and the concentration of IL-2 was evaluated by enzyme-linked immunosorbent assay. Cells from 12 donors were assessed. Data are the median percentage relative to uninfected cells. NA, not activated. *P < .05; ***P < .001 (Mann–Whitney U test). B, Histograms showing the expression of CD25 after 24 hours of activation (gray, isotype control). A representative experiment of n = 5 is shown. The lower part shows the median fluorescence intensity (MFI) of CD25 surface expression obtained from cells incubated with *H. pylori*, *H. pylori* CHOL, *H. pylori*D_{ggt}, or *H. pylori*D_{vacA} for 7 days. Cells from 5 donors were assessed. The graph depicts the percentage of MFI values relative to uninfected cells. NA, not activated. *P < .05; **P < .006 (Mann–Whitney U test). C) Expression of CD69 after 24 hours of activation (gray, isotype control); shown is a representative experiment of n = 6 (upper panel). Median MFI values of cells incubated with *H. pylori*, *H. pylori* CHOL, *H. pylori*D_{ggt}, or *H. pylori*D_{vacA} for 24 hours are shown. The graph depicts the percentage MFI relative to uninfected cells. Abbreviation: NA, not activated. *P < .05; **P < .006 (Mann–Whitney U test).
modulated by cholesterol coating of *H. pylori* nor dependent on VacA or GGT virulence factors (Figure 4B). Cells from 10 of 12 donors produced detectable levels of IL-6 upon activation ranging from 14 to 790 pg/mL. *H. pylori* enhanced the release of IL-6 under all conditions tested. Cholesterol coating or the absence of VacA or GGT had no effect on this *H. pylori*-enhanced IL-6 secretion (Figure 4C). Moreover, despite proliferation of CD4⁺ T cells being suppressed by the bacteria (see below), IL-6 levels were significantly increased after 7 days of incubation with *H. pylori* (data not shown).

These data show that although IL-17A production, presumably by Th17 cells, is not modulated by *H. pylori*, T-cell–dependent TGF-β1 production was inhibited and IL-6 secretion was stimulated. In contrast to type 1 and 2 related cytokines, neither cholesterol coating nor VacA or GGT played a role in the modulation of TGF-β1 and IL-6, suggesting the existence of yet another set of factors targeting this arm of CD4⁺ T-cell responses.

**Cholesterol α-Glucosyltransferase–Deficient *H. pylori* at Low MOI Lack Antiproliferative Activity on Human T Cells**

Previous work has implicated VacA and GGT in the *H. pylori*-induced blockage of T-cell proliferation, the latter factor exerting a more potent effect [6, 7]. Given the modulation of GGT- and VacA-mediated effects on cytokines by bacterial cholesterol content, we determined whether cholesterol coating of *H. pylori* would alter the proliferation of human CD4⁺ T cells. CFSE-stained CD4⁺ T cells were incubated with wild-type *H. pylori* or cholesterol-coated *H. pylori* prior to activation. In accordance with the results of previous work [25], *H. pylori* inhibited proliferation of primary T cells; however, this effect was dependent on GGT but independent on VacA, contradicting results of previous studies that used purified VacA [7] (Figure 5). Inhibition of T-cell proliferation was MOI-dependent, and consistent with our findings here, the effect of GGT was significantly modulated by bacterial cholesterol levels (Figure 5C), in particular at lower MOIs, which are probably more physiologically relevant.

Most of the cholesterol *H. pylori* requires for optimal growth becomes glucosylated through the action of a cholesterol α-glucosyl transferase encoded by open reading frame HP0421 [26]. In a mouse model of *H. pylori* infection, HP0421-deficient mutants are readily phagocytosed by antigen-presenting cells enhancing proliferation of specific T cells [9]. Therefore, we measured the proliferation of T cells upon exposure to an isogenic *H. pylori* strain lacking cholesterol α-glucosyl transferase (*H. pylori Δ421*) [9]. At high MOIs, T-cell proliferation was strongly inhibited by the mutant; however, this inhibitory effect was rapidly attenuated as MOI decreased (Figure 5B). The mutation had an effect similar to coating wild-type bacteria with cholesterol (Figure 5C). However, coating *H. pylori Δ421* with cholesterol did not modulate the inhibition of proliferation (Figure 5C). Similar results were obtained with a mutant of the gene HP0499, which
is also involved in the synthesis of cholesterol α-glucosides (data not shown). These data provide the first evidence that cholesterol α-glucosyl transferase activity is required for *H. pylori*-mediated direct inhibition of human CD4+ T-cell proliferation.

**DISCUSSION**

To date, 2 *H. pylori* virulence factors, VacA and GGT, have been implicated in directly suppressing human T cells. Purified VacA

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**Table 1. Apoptosis of CD4+ T Cells Induced by *H. pylori* Is Not Modulated by VacA, GGT, Alpha-Glucosyl Transferase or Cholesterol Loading**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Donor 1</th>
<th>Donor 2</th>
<th>Donor 3</th>
<th>Donor 4</th>
<th>Donor 5</th>
<th>Mean (SD)</th>
</tr>
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<tr>
<td>Not activated</td>
<td>6.3</td>
<td>7.3</td>
<td>4.9</td>
<td>3.1</td>
<td>5.3</td>
<td>5.4 (1.6)</td>
</tr>
<tr>
<td>Activated without bacteria</td>
<td>9.3</td>
<td>8.1</td>
<td>11.7</td>
<td>3.9</td>
<td>5.5</td>
<td>7.7 (3.1)</td>
</tr>
<tr>
<td><em>H. pylori</em></td>
<td>62.2</td>
<td>45.7</td>
<td>54.2</td>
<td>60.6</td>
<td>62.8</td>
<td>57.1 (7.2)</td>
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<td>64.5</td>
<td>50.6</td>
<td>61.4</td>
<td>67.7</td>
<td>75.4</td>
<td>63.9 (9.1)</td>
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<tr>
<td><em>H. pylori</em> Δ421</td>
<td>54.7</td>
<td>51.2</td>
<td>53.5</td>
<td>57.8</td>
<td>60.8</td>
<td>55.6 (3.7)</td>
</tr>
<tr>
<td><em>H. pylori</em> Δ421 CHOL</td>
<td>56.8</td>
<td>59.8</td>
<td>66.0</td>
<td>62.5</td>
<td>72.1</td>
<td>63.4 (5.9)</td>
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<td><em>H. pylori</em> ΔvacA</td>
<td>54.6</td>
<td>66.5</td>
<td>74.1</td>
<td>77.7</td>
<td>85.7</td>
<td>71.7 (11.8)</td>
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<td><em>H. pylori</em> Δggt</td>
<td>60.0</td>
<td>64.3</td>
<td>64.8</td>
<td>64.5</td>
<td>86.5</td>
<td>68.0 (10.5)</td>
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Human CD4+ T cells were incubated with *Helicobacter pylori*, cholesterol-coated *H. pylori* (*H. pylori* CHOL), a mutant strain lacking cholesterol α-glucosyl transferase (*H. pylori* Δ421), cholesterol-coated *H. pylori* Δ421 (*H. pylori* Δ421 CHOL), a mutant strain lacking γ-glutamyl transpeptidase (*H. pylori* Δggt), or a mutant strain lacking vacuolating exotoxin (*H. pylori* ΔvacA) at a multiplicity of infection of 50 for 2 hours and then activated with anti-CD3 and anti-CD28. Apoptosis was evaluated by fluorescence-activated cell sorting after 24 hours. No significant differences were found after treatment between *H. pylori*, *H. pylori* CHOL, and the mutants (analysis of variance test).

**Figure 3.** Interdependent effects of *Helicobacter pylori* virulence factors on T-helper 1 and T-helper 2 type cytokine release in activated human T cells. Human CD4+ T cells were incubated with *H. pylori*, cholesterol-coated *H. pylori* (*H. pylori* CHOL), mutant *H. pylori* lacking γ-glutamyl transpeptidase (*H. pylori* Δggt), or mutant *H. pylori* lacking vacuolating exotoxin (*H. pylori* ΔvacA) at a multiplicity of infection of 50 for 2 hours and then activated with anti-CD3 and anti-CD28. Supernatants were harvested after 24 hours, and the concentration of interleukin 10 (IL-10) (A), interleukin 4 (IL-4) (B), interferon γ (IFN-γ) (C), or tumor necrosis factor α (TNF-α) (D) was evaluated by enzyme-linked immunosorbent assay. Data are the median percentage relative to uninfected cells. NA, not activated. *P < .05 (Mann–Whitney U test). **P < .007.
has been shown to inhibit IL-2 production in human [8] but not murine T cells [28] that lack a cognate receptor [29]. Although the cholesterol/cholesterol α-glucosyl content of the bacteria had a profound effect on antigen presentation and T-cell responses in mice [9], its effect on human cells remained to be determined. Here, we demonstrate that bacterial cholesterol/cholesterol α-glucoside content constitute a third molecular component that synergises and/or modifies the activity of VacA and GGT to modulate human CD4⁺ T-cell responses.

Previous studies of H. pylori factors that inhibited T-cell responses have focused on secreted molecules, such as VacA and GGT, reasoning these may preferentially be encountered by T cells in the inflamed gastric tissue. However, H. pylori is also present deeper within infected tissues [30], and similar to other gram-negative bacteria, it sheds outer membrane vesicles. These vesicles are considered to be pathologically relevant mimicks of the bacterial membrane, carrying virulence factors [31] that can act at a distance [32, 33]. Thus, investigating the direct interaction of H. pylori with T cells at low MOIs using whole bacteria, as reported here, likely reflects physiological conditions.

Conditioned culture supernatants containing GGT or recombinant GGT have been shown to abolish T-cell proliferation [6], in a mechanism dependent on GGT enzymatic activity and, potentially, the modulation of the GTPase Ras signaling pathway, leading to cell cycle arrest. However, the process remains ill defined and may also be linked to the anti-proliferative role proposed for endogenous human GGT expressed by activated T cells [34]. Our results confirmed and extended previous findings that GGT blocks proliferation but has little or no significant modulating activity on additional response parameters such as secretion of pro- and anti-inflammatory cytokines. Furthermore, using whole mutant bacteria, we detected an additional inhibitory effect of GGT on CD25 expression. Most importantly, our findings indicate that the dramatic GGT-specific antiproliferative activity was subject to modulation by the bacterial cholesterol/cholesterol α-glucoside content, in particular at lower, likely more physiologically relevant MOIs. Cholesterol glucosides are thought to be characteristic of H. pylori because they are rarely found in other bacteria [35]. It has been proposed

Figure 4. Vacuolating exotoxin (VacA), γ-glutamyl transpeptidase (GGT), and cholesterol independent modulation of T-helper 17 related cytokine release in human CD4⁺ T cells. Human CD4⁺ T cells were incubated with Helicobacter pylori, cholesterol-coated H. pylori (H. pylori CHOL), a mutant strain lacking GGT (H. pylori Δggt), or a mutant strain lacking VacA (H. pylori ΔvacA) at a multiplicity of infection of 50 for 2 hours and then activated with anti-CD3 and anti-CD28. Supernatants were harvested after 24 hours, and the concentration of interleukin 17a (IL-17A) (A), transforming growth factor β1 (TGF-β1) (B), or interleukin 6 (IL-6) (C) was evaluated by enzyme-linked immunosorbent assay. Data are the median percentage relative to uninfected cells. NA, not activated. *P < .05 (Mann–Whitney U test). **P < .005, ***P < .001
that *H. pylori* controls the availability of cholesterol glucosylated phospholipids in the cell wall according to environmental changes [35]. Dendritic cell responses are modulated by *H. pylori* through phase variation of lipopolysaccharides [36]. Here, we show the first evidence that *H. pylori* directly manipulates host T-cell responses through changes in cholesterol/cholesterol α-glucoside content.

Purified VacA also inhibits CD4⁺ T-cell responses after polyclonal in vitro stimulation. This effect depends on the VacA allele present because only some forms bind to the leukocyte-specific receptor CD18 [29]. At high concentrations, VacA inhibited proliferation and expression of early surface activation markers such as CD25, as well as IL-2 production. Similar to cyclosporine or FK506, VacA inhibits the calcium-dependent activation of

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**Figure 5.** Inhibition of T-cell proliferation by cholesterol α-glucosyltransferase from *Helicobacter pylori*. A, Carboxyfluorescein succinimidyl ester (CFSE)–stained human CD4⁺ T cells were incubated with wild-type *H. pylori*, a mutant strain lacking γ-glutamyl transpeptidase (*H. pylori Δggt*), or a mutant strain lacking vacuolating exotoxin (*H. pylori ΔvacA*) at a multiplicity of infection (MOI) of 50 for 2 hours and then activated with anti-CD3 and anti-CD28 for 7 days. Shown is a representative experiment of *n* = 9. B, CFSE-stained human CD4⁺ T cells were incubated with wild-type *H. pylori* or a mutant strain lacking cholesterol α-glucosyl transferase (*H. pylori Δ421*) at MOI of 10, 20, or 50 for 2 hours and then activated with anti-CD3 and anti-CD28 for 7 days. Shown is the percentage of proliferating cells for a representative experiment of *n* = 9. C, CFSE-stained human CD4⁺ T cells were incubated with wild-type *H. pylori* or *H. pylori Δ421* uncoated (upper panels) or coated with cholesterol (CHOL) (lower panels) at MOI of 10, 20, or 50 for 2 hours and then activated with anti-CD3 and anti-CD28 for 7 days. NA, not activated. Data are expressed as a percentage of proliferating cells. Upper panels, *P* < .05, ***P* < .001 (analysis of variance Tukey posttest); lower panels, *P* < .05 (Wilcoxon test).
In order to persist within its host, the Helicobacter pylori (H. pylori) infection influences a variety of cytokine production levels. Our study, which compared VacA-deficient mutants with parental bacteria expressing a CD18- and ggt1, classified cytokine production as either pro-inflammatory (type I) or anti-inflammatory (type II). The table below summarizes the results:

<table>
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<th>Strain</th>
<th>Prolif.</th>
<th>CD69</th>
<th>CD25</th>
<th>IL-2</th>
<th>Th1</th>
<th>Th17</th>
<th>IL-4</th>
<th>IL-6</th>
<th>IL-10</th>
<th>TGF-β</th>
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<td>Down</td>
<td>Down</td>
<td>Down</td>
<td>Down</td>
<td>None</td>
<td>None</td>
<td>Up</td>
<td>Down</td>
<td>Down</td>
</tr>
<tr>
<td>H. pylori ΔvacA</td>
<td>Down</td>
<td>None</td>
<td>None</td>
<td>None</td>
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</tbody>
</table>

The table shows that H. pylori infection results in a downregulation of cytokine production compared with activated CD4+ T cells. VacA and GGT, cholesterol/cholesterol ß-glucosides and other factors target specific aspects of T-cell responses summarized in Table 2. The findings are consistent with previous observations that H. pylori supports T-cell–dependent inflammation by maintaining high levels of TNF-α and IL-17A while suppressing IL-10 and TGF-β. In addition, up-regulation of IL-6 and down-regulation of IL-4 by H. pylori may be linked to the development of gastric cancer [24, 42, 43], heart disease [44, 45], and the inverse association between H. pylori infection and asthma [46-48]. Thus, the interplay between H. pylori and human CD4+ T cells appears to be a key event in the establishment of a chronic infection that affects one-half of the world’s population and is linked to a number of comorbidities [1].

**Notes**

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