Helicobacter pylori Coinfection Is Associated With Decreased Markers of Immune Activation in ART-Naive HIV-Positive and in HIV-Negative Individuals in Ghana

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Background. *Helicobacter pylori* coinfection in human immunodeficiency virus (HIV) patients has been associated with higher CD4+ cell counts and lower HIV-1 viral loads, with the underlying mechanisms being unknown. The objective of this study was to investigate the impact of *H. pylori* infection on markers of T-cell activation in HIV-positive and HIV-negative individuals.

Methods. In a cross-sectional, observational study, HIV patients (n = 457) and HIV-negative blood donors (n = 79) presenting to an HIV clinic in Ghana were enrolled. Data on clinical and sociodemographic parameters, CD4+/CD8+ T-cell counts, and HIV-1 viral load were recorded. *Helicobacter pylori* status was tested using a stool antigen test. Cell surface and intracellular markers related to T-cell immune activation and turnover were quantified by flow cytometry and compared according to HIV and *H. pylori* status.

Results. *Helicobacter pylori* infection was associated with decreased markers of CD4+ T-cell activation (HLA-DR+CD38+CD4+; 22.55% vs 32.70%; $P = .002$), cell proliferation (Ki67; 15.10% vs 26.80%; $P = .016$), and immune exhaustion (PD-1; 32.45% vs 40.00%; $P = .005$) in 243 antiretroviral therapy (ART)–naive patients, but not in 214 patients on ART. In HIV-negative individuals, *H. pylori* infection was associated with decreased frequencies of activated CD4+ and CD8+ T cells (6.31% vs 10.40%; $P = .014$ and 18.70% vs 34.85%, $P = .006$, respectively).

Conclusions. Our findings suggest that *H. pylori* coinfection effectuates a systemic immune modulatory effect with decreased T-cell activation in HIV-positive, ART-naive patients but also in HIV-negative individuals. This finding might, in part, explain the observed association of *H. pylori* infection with favorable parameters of HIV disease progression.

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Keywords. HIV/AIDS; *Helicobacter pylori*; immune activation; sub-Saharan Africa.

Human immunodeficiency virus (HIV) infection causes depletion of CD4+ T cells. Ongoing HIV viral replication results in progressive depletion of CD4+ T cells, expansion of CD8+ T cells, and, correspondingly, a low CD4+/CD8+ cell ratio [1, 2]. Antiretroviral therapy (ART) has led to a clear decline in morbidity and mortality among HIV-infected patients, mainly through its sustained suppression of HIV replication. However, treatment-mediated immune reconstitution is often incomplete, even after years of viral suppression [3]. Inflammation and T-cell activation remain elevated and CD4+ T-cell counts often fail to achieve normal levels [4]. Persistent immune activation is a hallmark

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of HIV infection, resulting in exhaustion of the regenerative capacities of the immune system and consecutively in immunodeficiency and AIDS [5]. Furthermore, numerous non-HIV–related complications such as cardiovascular diseases, osteoporosis, neurocognitive decline, and non-AIDS associated cancer can be considered direct or indirect consequences of a chronic inflammatory status [6]. Several factors contribute to the chronic, generalized immune activation observed in HIV-infected individuals. In addition to HIV itself, HIV-mediated breakdown of the gut mucosal barrier and subsequent chronic exposure to intestinal microbial products such as lipopolysaccharide, exposure to other pathogens such as cytomegalovirus, or pyroptosis-induced cell death with subsequent release of proinflammatory cytokines might contribute to immune activation and HIV pathology [7, 8].

Helicobacter pylori is a gram-negative bacterium with a high prevalence of up to 85% in sub-Saharan Africa, the region that is also most affected by the HIV epidemic [9]. *Helicobacter pylori* is usually acquired during childhood and persistently colonizes the human stomach or duodenum [9]. The infection may contribute to the development of chronic gastritis, which can lead to peptic ulcer disease, gastric adenocarcinoma, and mucosa-associated lymphoid tissue lymphoma [10].

There is evidence that chronic *H. pylori* infection modulates the systemic immune response. Protective effects of *H. pylori* against the development of allergic asthma, inflammatory bowel diseases, and tuberculosis infection have been demonstrated [11–14]. It was shown that *H. pylori* infection is associated with enhanced interferon-gamma (IFN- γ) responses to tuberculosis [15]. On the other hand, *H. pylori* has been linked to a number of extraintestinal pathologies, including cardiovascular diseases, chronic urticaria, rosacea, Sjögren syndrome, and idiopathic thrombocytic purpura, in addition to pathologic changes of the gastric mucosa [16]. Furthermore, *H. pylori*–induced hypochlorydria leads to changes in the gastric microbiota composition, potentially resulting in an altered intestinal colonization and possible associations with pathogens such as *Shigella* or *Vibrio cholerae* [17, 18].

Potential regulatory properties on systemic immune response, especially on the activation of peripheral T lymphocytes, could be of particular interest for HIV pathology, since antiinflammatory drugs have been shown to be associated with a more favorable course of HIV disease [19, 20]. However, only a few studies with smaller sample sizes have investigated markers of activated peripheral regulatory T cells (Tregs) in *H. pylori*–positive persons, and those studies found inconsistent conclusions [21–23]. Furthermore, no data on associations between *H. pylori* and the systemic immune response and chronic inflammation in people living with HIV have been published to date. Hence, in the present study, we investigated the association between *H. pylori* infection and markers of immune activation (HLA-DR+CD38+), cell proliferation (Ki67), immune senescence (CD57), and immune exhaustion (PD-1) on T-cell subsets in a large HIV cohort and in HIV-negative controls in Ghana, West Africa.

METHODS

Study Design and Study Population

In this cross-sectional observational study, consecutive HIV-infected patients presenting to the HIV outpatient clinic and HIV-negative blood donors serving as controls were recruited between November 2011 and November 2012 at the Komfo Anokye Teaching Hospital, a tertiary referral hospital in Kumasi, Ghana. The appropriate ethics committees in Ghana and Germany approved the study. Written informed consent was obtained from all participants before enrollment in the study.

Data Collection and Laboratory Methods

Trained study personnel collected demographic and clinical data using a standardized questionnaire. Blood samples were collected in EDTA tubes for analysis of CD4+/CD8+ T-cell counts, using a FACSCalibur flow cytometer (Becton Dickinson, Mountain View, California). Peripheral blood mononuclear cells (PBMCs) were isolated by centrifugation of heparinized venous blood on a Ficoll/Hypaque (Biocoll Seperating Solution, Biochrom AG, Berlin, Germany) density gradient. Cells were washed in phosphate-buffered saline and resuspended in Roswell Park Memorial Institute 1640 medium (both Gibco Invitrogen, Carlsbad, California) supplemented with heat-inactivated fetal calf serum (Biochrom AG, Berlin, Germany). PBMCs were cryopreserved and shipped to Germany on liquid nitrogen. EDTA plasma and native stool samples were freshly frozen and stored at −80°C until being transported to Germany on dry ice.

Stool was tested for *H. pylori* using the RidaScreen FemtoLab *H. pylori* stool antigen test (R-Biopharm AG, Darmstadt, Germany). HIV-1 viral load was measured using the RealTime HIV-1 polymerase chain reaction system (Abbott Diagnostics, Wiesbaden, Germany). The same tests, except viral load analysis, were applied to cases and controls.

Cell surface markers for immune activation and immune exhaustion/function were stained using a fluorochrome-conjugated mouse anti-human monoclonal antibody combination in a single panel: anti-CD3-APC-H7, anti-CD4-V500, anti-CD8-PerCP, anti-HLA-DR-FITC, anti-CCR7-Alexa-Flour-647 (CD197) (BD Biosciences, Heidelberg, Germany) and anti-CD38-PE-Cy7, anti-PD-1-V421, anti-CD57-PE, anti-CD45RA-Alexa-Flour-700 (Biolegend, Fell, Germany). In a second panel, cell surface markers of immune regulation and cell proliferation/cell turnover were stained using anti-CD3-PerCP, anti-CD4-Pacific Blue, anti-CD8-Alexa-Flour-700, and anti-CD25-PE-Cy7 (BD Biosciences, Heidelberg, Germany). The stained cells were fixated and permeabilized (FoxP3 staining buffer set, eBioscience,

Frankfurt a. M., Germany) for intracellular staining using anti-FOX-P3-PE (Biolegend, Fell, Germany) and anti-Ki-67-Alexa-Flour-647 (BD Biosciences, Heidelberg, Germany). Flow cytometric data were acquired using the LSRII flow cytometer (BD Biosciences, Heidelberg, Germany), and acquisition was set to 500 000 cells/sample for panel 1 and 1 000 000 cells/sample for panel 2. Compensation was conducted with antibody capture beads (BD CompBeads Set Anti-Mouse Ig, κ, BD Biosciences, Heidelberg, Germany), stained separately with the individual fluorochrome-conjugated monoclonal antibodies used in all samples. Flow cytometry measurements were performed in runs of 20 samples, each including samples of HIV-positive individuals and HIV-negative controls. Cutoffs for CD38 and HLA-DR expression were defined in an HIV-negative sample on the naive (CCR7+CD45RA+) T-cell population, typically expressing CD38, but only negligible amounts of HLA-DR, and uniformly applied to all samples of 1 run (Figure 1). A fluorescent minus one control experiment was done to confirm the gating strategy. Flow cytometry measurements were analyzed using FlowJo version 9.6.2 (Tree Star, San Carlos, California). The operator was blinded to participants' clinical and laboratory data. All samples of HIV-positive individuals and HIV-negative controls were processed according to the same protocols.

Statistical Analyses

Continuous variables were expressed as mean ± standard deviation or median (interquartile range [IQR]) and compared using the unpaired Student *t* test or the Wilcoxon rank sum test. Proportions were compared using either the χ^2 test or Fisher exact test as appropriate. A multivariable linear regression model was used to assess the association between the continuous outcome variables HLA-DR+CD38+ as activation marker of CD4+/CD8+ T cells and other laboratory, clinical, and demographic parameters. After assessing the Pearson correlation between the frequency of HLA-DR+CD38+CD4+ and HLA-DR+CD38+CD8+ T cells and age, gender, time since HIV diagnosis, use of co-trimoxazole or rifampicin in the last 6 months, and *H. pylori* status, only parameters with a *P* value ≤.1 were included in a linear multivariate regression model. The parameters CD4+ and CD8+ T-cell count and HIV-1 viral load, being directly linked to T-cell activation, were excluded. However, the linear multivariate regression model was alternatively calculated including those parameters. All *P* values were 2-sided, and *P* values <.05 were considered statistically significant. Statistical analyses were conducted with R 2.15.0 (R Foundation for Statistical Computing, Vienna, Austria).

RESULTS

Cohort Characteristics

A total of 1095 HIV-positive patients and 107 HIV-negative blood donors were recruited for the original cohort study, which sought to access the sociodemographic and clinical determinants of *H. pylori* coinfection among HIV-infected and non-infected individuals. Flow cytometry data were available for 457 (41.7%) HIV-positive and 79 (73.8%) HIV-negative participants. Compared with the original cohort, HIV-positive and negative patients with available flow cytometry data were not different from those without flow cytometry data in terms of gender, age, CD4+ cell count, or viral load.

Among patients with available flow cytometry data, HIV-positive individuals were older (40.4 vs 33.4 years; $P < .001$), had a lower CD4+/CD8+ ratio (0.42 vs 2.18; $P < .001$), and a lower prevalence of *H. pylori* infection (56.2% vs 87.3%; $P < .001$) compared with HIV-negative individuals. Markers of immune activation, senescence, exhaustion, and cell turnover on T-cell subsets differed significantly between HIV-positive and HIV-negative individuals (Table 1). Within the group of HIV-positive participants, approximately half of the participants (46.8%) were receiving ART; 53.2% were ART naive. Patients on ART were more likely to be female (81.3% vs 70.0%; $P = .005$), had a higher mean body mass index (24.3 vs 22.3; $P < .001$), a higher median CD4+ T-cell count (483 [IQR, 301–671] vs 269 [IQR, 105–448] cells/μL; $P < .001$), and were more frequently coinfected with *H. pylori* (62.2% vs 51.0%; $P = .017$) compared with ART-naive patients.

Markers of Immune Activation According to *H. pylori* Status

No differences in demographics were observed within the subgroups when compared according to *H. pylori* status (Table 2). Within the group of HIV-positive, ART-naive participants, those with *H. pylori* coinfection had significantly higher median CD4+ T-cell counts (312 [IQR, 135–484] vs 224 [IQR, 79–426] cells/μL; $P = .024$) and lower median HIV-1 viral loads (4.82 vs 5.18 log10 copies/mL; $P = .004$). Frequencies of HLA-DR+CD38++CD4+ (22.55% vs 32.70%; $P = .002$), Ki67+CD4+ (15.1% vs 26.8%; $P = .016$), PD-1+CD4+ (32.45% vs 40.0%; $P = .005$), as well as Ki67+CD8+ (10.3% vs 16.6%; $P = .031$) and PD-1+CD8+ (36.15% vs 41.50%; $P = .012$) T cells were lower in individuals with vs without *H. pylori* coinfection (Table 3; Figure 2).

In the subgroup of HIV-positive participants on ART, *H. pylori* infection was associated with higher CD4+/CD8+ ratios (0.59 vs 0.43; $P = .010$) and a trend toward lower frequencies of CD25+FoxP3+CD4+ T cells (1.83% vs 2.44%; $P = .059$). No differences regarding markers for immune activation, exhaustion, or proliferation could be detected between *H. pylori*–positive vs *H. pylori*–negative individuals (Table 3). Interestingly, frequencies of CD4+ and CD8+ T cells expressing the activation markers HLA-DR+CD38+ were lower in HIV-negative blood donors with *H. pylori* compared with those without *H. pylori* infection (median 6.31 vs 10.40; $P = .014$ and 18.70 vs 34.85; $P = .006$, respectively) (Figure 2, Supplementary Figure 1). They also had a trend toward higher CD4+/CD8+ ratios (2.22 [IQR, 1.79–3.05] vs 1.77 [IQR, 1.01–2.38]; $P = .087$; Table 3).

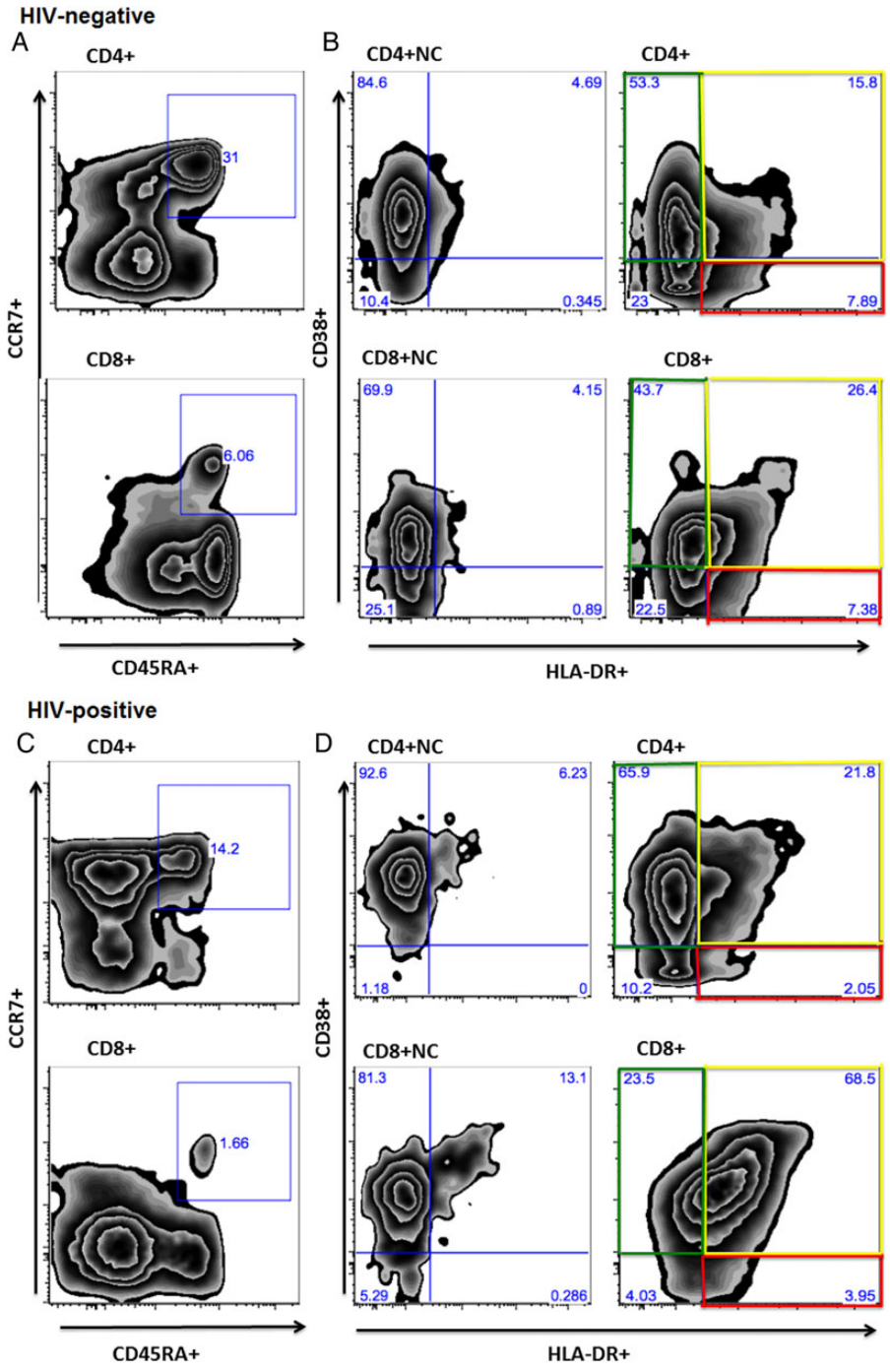

Figure 1. Gating strategy for HLA-DR and CD38 expression on peripheral CD4+ and CD8+ T cells of human immunodeficiency virus (HIV)–negative (panels *A* and *B*) and HIV-positive (panels *C* and *D*) individuals. Cutoffs for CD38 and HLA-DR expression were defined in an HIV-negative sample on the respective naive (CCR7+CD45RA+) T-cell population (panel *B*), typically expressing CD38 and only negligible amounts of HLA-DR, and uniformly applied to all samples of 1 run (panels *C* and *D*).

Independent Predictors of Immune Activation

In the multivariate linear regression analysis, a negative *H. pylori* status was identified as an independent risk factor for CD4+ T-cell activation in HIV-positive, ART-naive participants ($P = .008$). In HIV patients receiving ART, female gender and months since diagnosis of HIV infection, but not *H. pylori* infection, were associated with immune activation. Negative *H. pylori* status was identified as the only independent risk factor

Table 1. Immunological Parameters According to Human Immunodeficiency Virus Status

Variable, Median (Interquartile Range)	HIV Positive, n = 457	HIV Negative, n = 79
CD4+ T-cell count/μL	380 (184–567)	957 (769–1134)**
CD8+ T-cell count/μL	854 (610–1335)	420 (309–617)**
CD4+/CD8+ T-cell ratio	0.42 (0.21–0.69)	2.18 (1.69–2.96)**
HLA-DR+CD38+CD4+ (%)	17.90 (10.10–31.90)	6.69 (4.96–9.43)**
Ki67+CD4+ (%)	11.90 (6.98–26.25)	4.77 (3.80–5.75)**
PD-1+CD4+ (%)	34.10 (23.40–48.50)	31.00 (20.15–35.95)*
CD57+CD4+ (%)	16.30 (9.47–27.70)	12.80 (8.46–24.95)
CD25+Foxp3+CD4+ (%)	2.53 (1.50–4.43)	1.59 (1.03–2.26)**
HLA-DR+CD38+CD8+ (%)	40.60 (27.30–54.50)	19.20 (15.50–28.05)**
Ki67+CD8+ (%)	10.03 (6.04–17.48)	4.70 (3.55–5.88)**
PD-1+CD8+ (%)	30.40 (18.70–43.60)	15.30 (9.54–20.60)**
CD57+CD8+ (%)	50.20 (39.60–61.00)	61.10 (42.65–72.10)**

Abbreviation: HIV, human immunodeficiency virus.

*$P < .01$; **$P < .001$.

for increased CD4+ ($P = .001$) and CD8+ ($P < .001$) T-cell activation in HIV-negative individuals (Table 4). Also, after adjustment for CD4+ T-cell count and HIV-1 viral load, *H. pylori* infection was independently associated with decreased CD4+ T-cell activation in ART-naive HIV-infected individuals and with CD4+ and CD8+ T-cell activation in HIV-negative individuals (Supplementary Table 1).

DISCUSSION

Data from epidemiologic studies suggest that the prevalence of *H. pylori* infection is clearly lower in HIV-positive compared with HIV-negative individuals and that it further declines with the progression of immunodeficiency in HIV-infected patients. *Helicobacter pylori* coinfection is also associated with higher CD4+ T-cell counts and lower HIV-1 viral loads [24].

Several mechanisms have been proposed to explain this association. First, *H. pylori* infection itself could exert an effect on the progression of, or the susceptibility to, HIV infection. Recently, the importance of gastrointestinal microbiota as a determinant for the systemic immune response has been recognized, and a number of extraintestinal, immune-related implications of *H. pylori* infection have been reported [16, 17, 25]. Other explanations include the more frequent use of antibiotics in HIV patients, in particular those with more advanced HIV disease, leading to inadvertent eradication of *H. pylori*. Results from our original cohort study could, however, not explain differences in *H. pylori* prevalence by more frequent use of antibiotics or socioeconomic status in HIV-positive patients.

Table 2. Cohort Characteristics According to *Helicobacter pylori* Status

	HIV Positive, Antiretroviral Therapy Naive		HIV Positive on Antiretroviral Therapy		HIV Negative	
Characteristic	*H. pylori* Positive, n = 124	*H. pylori* Negative, n = 119	*H. pylori* Positive, n = 133	*H. pylori* Negative, n = 81	*H. pylori* Positive, n = 69	*H. pylori* Negative, n = 10
Female gender, n (%)	86 (69.36)	84 (70.59)	108 (81.20)	66 (81.48)	43 (64.18)	8 (80.0)
Age (y), mean ± SD	39.46 ± 9.58	41 ± 9.68	41 ± 8.50	40 ± 8.59	33 ± 13.32	33 ± 13.88
Body mass index (kg/m²), mean ± SD	22.38 ± 4.01	22.2 5 ± 4.88	24.59 ± 5.52	23.80 ± 4.61	24.55 ± 5.22	24.77 ± 6.13
Anti-tuberculosis treatment, n (%)	7 (5.65)	11 (9.24)	1 (0.75)	1 (1.24)	NA	NA
Co-trimoxazole, n (%)	27 (21.77)	39 (32.77)	33 (24.81)	19 (23.46)	NA	NA
Other antibiotics, n (%)	0	0	0	0	NA	NA
Months since diagnosis, median (interquartile range)	0.0 (0.0–2.8)	0.0 (0.0–3.0)	55.0 (26.5–83.5)	51.0 (27.25–75.5)	NA	NA

No significant differences were detected between *H. pylori*–positive vs *H. pylori*–negative individuals within each subgroup.

Abbreviations: HIV, human immunodeficiency virus; NA, not applicable; SD, standard deviation.

Table 3. Immunological Parameters According to *Helicobacter pylori* Status

Variable, median (interquartile range or %)	HIV Positive, Antiretroviral Therapy Naive		HIV Positive on Antiretroviral Therapy		HIV Negative	
	H. pylori positive, n = 124	*H. pylori* negative, n = 119	*H. pylori* positive, n = 133	*H. pylori* negative, n = 81	*H. pylori* positive, n = 69	*H. pylori* negative, n = 10
CD4+ T-cell count/μL	312 (135–484)	224 (79–426)*	505 (332–719)	448 (296–590)	958 (787–1169)	793 (737–980)
CD8+ T-cell count/μL	1186 (872–1739)	1326 (887–2034)	1462 (1092–2043)	1396 (1072–2072)	411 (304–548)	585 (412–811)
CD4+/CD8+ T-cell ratio	0.34 (1.75–5.49)	0.19 (0.09–0.42)**	0.59 (0.41–0.93)	0.43 (0.32–0.81)*	2.22 (1.79–3.05)	1.77 (1.01–2.38)
Viral load, log10 copies/mL	4.82 (4.09–5.43)	5.18 (4.57–5.67)**	1.59 (1.59–2.19)	1.59 (1.59–1.83)	NA	NA
HLA-DR+CD38+CD4+ (%)	22.55 (13.70–34.93)	32.70 (18.65–41.25)**	12.90 (7.30–20.60)	11.90 (7.76–18.90)	6.31 (4.94–8.15)	10.40 (8.67–15.15)*
Ki67+CD4+ (%)	15.10 (8.33–32.10)	26.80 (15.10–49.00)*	7.96 (5.48–12.00)	9.33 (6.98–13.50)	4.74 (3.75–5.73)	5.09 (4.26–6.51)
PD-1+CD4+ (%)	32.45 (23.33–48.73)	40.00 (28.35–55.30)**	31.80 (21.30–44.40)	31.70 (18.30–46.40)	30.90 (19.80–35.20)	35.95 (25.03–37.28)
CD57+CD4+ (%)	13.90 (9.39–25.73)	18.30 (9.70–29.50)	16.10 (9.32–26.90)	16.70 (10.00–30.30)	12.80 (8.16–24.50)	14.25 (11.25–39.78)
CD25+Foxp3+CD4+ (%)	2.54 (1.52–4.54)	3.28 (1.79–6.83)	1.83 (1.15–3.76)	2.44 (1.64–4.83)	1.59 (1.12–2.23)	1.46 (0.80–2.30)
HLA-DR+CD38+CD8+ (%)	49.25 (39.08–62.10)	52.90 (43.35–63.95)	27.90 (20.00–40.60)	26.00 (18.10–36.40)	18.70 (15.30–26.20)	34.85 (22.13–44.58)**
Ki67+CD8+ (%)	10.30 (7.88–15.90)	16.60 (11.20–23.10)*	6.04 (4.02–8.53)	9.27 (4.29–17.13)	4.58 (3.47–5.92)	5.19 (4.47–5.82)
PD-1+CD8+ (%)	36.15 (23.85–45.83)	41.50 (28.85–53.25)*	22.60 (14.50–34.80)	22.30 (16.10–32.80)	15.10 (9.57–20.40)	17.60 (6.48–23.175)
CD57+CD8+ (%)	44.85 (36.30–55.43)	45.80 (35.25–57.35)	54.40 (45.80–65.10)	52.80 (43.80–62.20)	60.00 (42.00–71.70)	66.65 (54.80–72.70)

Abbreviations: HIV, Human Immunodeficiency virus; NA, not applicable.

* $P < .05$; ** $P < .01$ compared between *H. pylori*–positive vs *H. pylori*–negative individuals within each subgroup.

There is no evidence that CD4+ T cells are needed for the maintenance of *H. pylori* infection or that the depletion of CD4+ T cells could lead to the loss of *H. pylori* infection. Rather, T-cell responses against *H. pylori* have been shown to be associated with gastric inflammation and protection against *H. pylori* infection [26]. The fact that the observed differences between individuals with vs without *H. pylori* infection were also observed in HIV-negative participants is another argument against this hypothesis.

It is known that the main target cells for HIV are activated CD4+ T lymphocytes, of which the majority is located in the lymphoid tissue of the gastrointestinal mucosa [7]. *Helicobacter pylori* might prevent the activation of CD4+ T cells for maintaining its own persistence in the gastric and duodenal mucosa via several mechanisms, thereby reducing the number of target cells susceptible for HIV infection and possibly slowing down the vicious circle of immune activation and HIV replication [27, 28].

The immune response to *H. pylori* infection is predominantly T-cell mediated, with Th1 and Th17 cells being major effectors [29]. *Helicobacter pylori* has evolved multiple mechanisms to evade adaptive immunity by interfering with antigen presentation and modulation of T-cell responses [30]. It has been shown that the *H. pylori* vacuolating toxin (VacA) directly inhibits T-cell activation by interfering with the maturation of dendritic cells and antigen presentation and by inhibiting activation-induced proliferation of T and B lymphocytes [30–33]. Apparently, *H. pylori* is able to induce Treg responses, while inhibiting Th17 responses [30, 34]. Treg cells are increased in the gastric mucosa of *H. pylori*–infected patients and attenuate the inflammatory response, among other mechanisms, by secreting the antiinflammatory cytokines transforming growth factor-β1 and interleukin-10 and thus facilitate the colonization of the stomach [21].

To date, it is not clear if those mechanisms are also relevant for the systemic immune response and thus could, in part, explain the decreased peripheral T-cell activation observed in this study. However, one study investigated the effect of *H. pylori* eradication on the cytokine profile of patients with chronic immune thrombocytopenia (cITP). Six months after eradication, those patients who achieved cITP remission showed a significant reduction in the concentrations of predominantly proinflammatory Th1- and Th17-associated cytokines and an increase in Treg- and Th2-associated cytokines [35]. Furthermore, it has recently been noted that *H. pylori* infection might trigger large intestinal microbiota changes, with possible implications for microbial translocation and immune activation [17, 25].

Helicobacter pylori infection, by modulating mucosal and systemic immunity, might influence susceptibility or the clinical course of other infections. Only 1 study has investigated the relationship between *H. pylori* seroprevalence and malaria incidence in Ugandan children; no evidence for a protective effect against malaria was found [36]. In contrast, a protective effect of

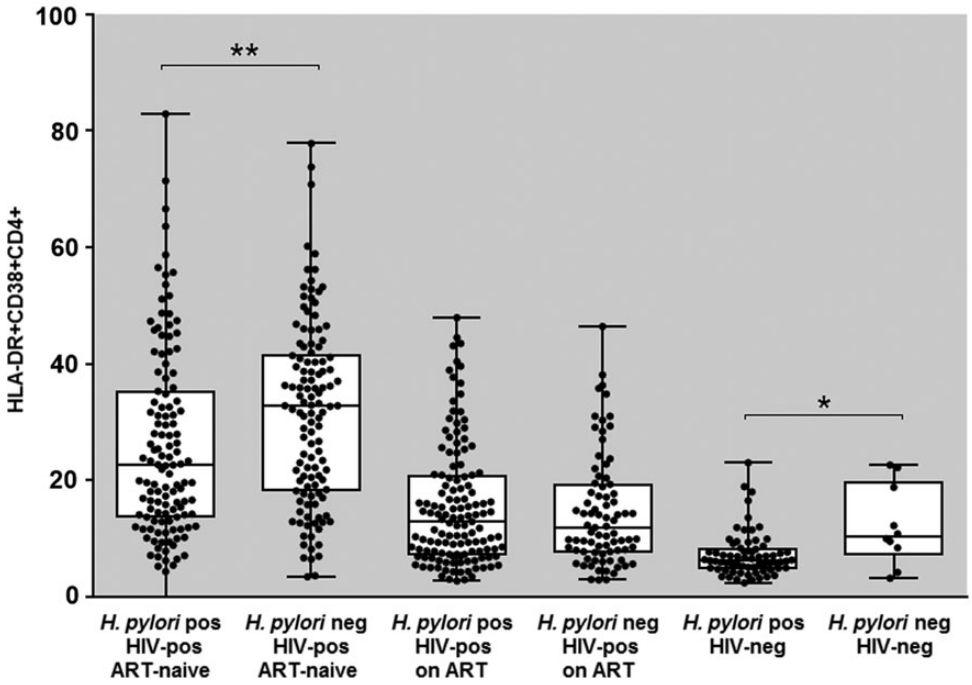

Figure 2. Boxplot scatter dot plot showing the proportion of activated CD4+ T cells (HLA-DR+CD38+CD4+) within the 3 subgroups of human immunodeficiency virus (HIV)–positive, antiretroviral therapy (ART)–naive patients, HIV-positive participants on ART and HIV-negative blood donors compared according to their *Helicobacter pylori* status. Intragroup comparisons were conducted using the Wilcoxon rank sum test, with *$P < .05$; **$P < .01$.

H. pylori against tuberculosis infection associated with enhanced IFN-γ responses has been reported in human tuberculosis case-contact cohorts and in monkeys that underwent a tuberculosis challenge [37].

Table 4. Univariate and Multivariate Analysis of Factors Associated With Increased Immune Activation

	HIV Positive, Antiretroviral Therapy Naive, n = 243				HIV Positive on ART, n = 214				HIV Negative, n = 79			
	Univariate		Multivariate		Univariate		Multivariate		Univariate		Multivariate	
Variable	*r*	*P* Value	β-Coef	*P* Value	*r*	*P* Value	β-Coef	*P* Value	*r*	*P* Value	β-Coef	*P* Value
Factors associated with increased HLA-DR+CD38+CD4+												
Female gender	−0.031	.636			0.072	.292			−0.050	.663		
Age, y	0.038	.552			−0.150	.029	0.104	.184	0.037	.747		
Anti-tuberculosis treatment	0.065	.315			0.070	.305			NA	NA		
Co-trimoxazole	0.009	.885			−0.074	.281			NA	NA		
Months since diagnosis	−0.137	.037	−0.134	.057	−0.290	<.001	-0.088	**<.001**	NA	NA		
Helicobacter pylori positive	−0.175	.006	−5.559	**.008**	0.044	.518			−0.359	.001	−4.923	**.001**
Factors associated with increased HLA-DR+CD38+CD8+												
Female gender	−0.074	.252			0.121	.078	4.997	**.049**	−0.138	.230		
Age, y	0.040	.534			−0.089	.193			−0.155	.179		
Anti-tuberculosis treatment	0.101	.115			0.094	.170			NA	NA		
Co-trimoxazole	0.096	.134			0.063	.361			NA	NA		
Months since diagnosis	−0.102	.120			−0.212	.002	−0.098	**.002**	NA	NA		
Helicobacter pylori positive	−0.075	.242			0.060	.382			−0.422	<.001	−14.098	**<.001**

The bold values represent $P < .05$.

Abbreviations: ART, antiretroviral therapy; β-Coef, multivariate linear regression coefficient (slope of regression line showing increase of outcome variable for every 1-unit increase in each predictor); HIV, human immunodeficiency virus; NA, not applicable; r, Pearson correlation coefficient.

This is the first study to systematically investigate the association between *H. pylori* infection and systemic immune activation in HIV-positive and HIV-negative individuals. Using multivariate regression analysis, *H. pylori* infection was associated with decreased markers of immune activation in CD4+ T cells and with decreased markers of immune exhaustion and cell turnover in CD4+ and CD8+ T cells in ART-naive HIV patients. Interestingly, *H. pylori* infection was also associated with decreased frequencies of activated CD4+ and CD8+ T cells in HIV-negative blood donors. This finding is remarkable considering the relatively small sample size of the HIV-negative control group and suggests that the observed correlation of *H. pylori* infection with decreased immune activation is not specific for HIV-infected individuals and that the observed association in HIV-infected participants is unlikely to be explained by confounders in the HIV-positive group. Furthermore, a higher level of T-cell activation in *H. pylori*–negative, HIV-uninfected individuals might potentially support the hypothesis that *H. pylori* infection decreases the susceptibility to HIV infection. Indeed, immune activation has been described as a risk factor for the acquisition of HIV infection in the CAPRISA 004 vaccination trial [38, 39]. In another trial, an association of CD8+ T-cell activation with increased risk of HIV infection was reported [40]. The authors noted that identifying causes for elevated innate immune activation could enable targeted prevention measures.

The failure to detect differences in the subgroup of HIV-infected individuals receiving ART might be explained by the markedly decreased baseline immune activation in those patients, together with the heterogeneity regarding duration and kind of ART. However, those patients with *H. pylori* infection had significantly higher CD4+/CD8+ ratios as an indicator for decreased immune activation compared with individuals without *H. pylori* coinfection.

There are limitations of our study to be mentioned. Most importantly, the causality of the observed associations cannot be established with the cross-sectional study design used. Longitudinal studies would be needed to explore the hypotheses that the risk of HIV acquisition is decreased in *H. pylori*–positive vs *H. pylori*–negative individuals and that *H. pylori* acquisition, respectively eradication, is associated with alterations in immune activation. The HIV-negative control group was smaller than the HIV-positive group, with a higher median age and most likely a lower risk for coinfections, making the intergroup comparison of immune activation problematic. However, the main focus of this study was the intragroup analysis of immune parameters according to *H. pylori* status. Overall, our findings support the hypothesis that *H. pylori* coinfection effectuates a systemic immune modulatory effect with decreased T-cell activation in HIV-positive, ART-naive patients and also in HIV-negative individuals. This might, in part, explain the observed association of *H. pylori* infection with favorable parameters of HIV disease progression and other extraintestinal effects.

The mechanisms of possibly beneficial immunomodulatory effects of *H. pylori* infection, which need to be characterized, might potentially represent a new therapeutic approach. At the same time, the high global burden of *H. pylori* infection warrants the development of vaccine or eradication strategies, emphasizing the definition of respective target groups.

Supplementary Data

Supplementary materials are available at *Clinical Infectious Diseases* online (http://cid.oxfordjournals.org). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

Notes

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