Eradication of *Helicobacter pylori* can facilitate immune reconstitution in HIV-1-infected immunological non-responders

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**ABSTRACT**

**Objective:** A significant number of HIV-1 patients experience poor immune reconstitution despite long-term viral suppression with highly active antiretroviral therapy (immunological non-responders). The aims of the present study were to determine whether eradication of *Helicobacter pylori* could facilitate a better immune reconstitution in these patients.

**Methods:** Forty-nine immunological non-responder HIV-1 patients were evaluated by 13C-urea breath test (UBT) for the presence of active *H. pylori* infection. They were all asymptomatic. The UBT was positive in 26 (53%) of them. Eleven patients (group 1) were treated with a combination of omeprazole 20 mg bid, amoxicillin 1 g bid and clarithromycin 500 mg bid for 14 consecutive days. Eight weeks later, successful eradication was proven by a repeat negative UBT in all 11 patients. The remaining 15 (group 2) refused the *H. pylori* eradication treatment. All 26 patients were followed for 24 months and evaluated for blood CD4 and CD8 cell counts and percentages and for plasma HIV-1 viral load.

**Results:** At the time of *H. pylori* diagnosis and eradication (baseline), CD4 and CD8 cell counts were similar in both study groups. All 11 *H. pylori* eradicated patients (group 1) had a significant increase in CD4 cell count starting 3 months and peaking 12–18 months after *H. pylori* eradication. Thereafter, CD4 levels gradually declined. Nevertheless, 24 months after triple therapy it was significantly higher than prior to *H. pylori* eradication. Parallel reciprocal changes were observed in CD8 cell counts. There were no significant changes in either CD4 or CD8 cell counts in group 2 patients. None of the patients of group 1 demonstrated virological failure, while four (26.7%) group 2 patients experienced virological failure requiring change of highly active antiretroviral therapy (HAART) regimen.

**Conclusion:** Triple therapy for *H. pylori* eradication is associated with a significant, although possibly transient immune reconstitution in HAART-treated HIV-1 patients with viral suppression without immunological response.

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1. Introduction

Highly active antiretroviral therapy (HAART) has dramatically improved the prognosis of patients infected with HIV-1. In the majority of HIV-1 infected patients under HAART, plasma viral load becomes undetectable (low detectable level, LDL) and CD4 cells increase to near normal levels over time. Nevertheless, in a portion of the patients, a discrepancy between plasma viral load and the immune status is observed. In this population, CD4 cell counts do not increase despite full plasma viral load suppression (immunological non-responders). Approximately one-third of patients receiving HAART for 5 years or more will not reach CD4 cell counts above 500 cells/mm³ despite continuous suppression of plasma HIV-1 RNA. Moreover, CD4 cell counts may remain < 200 cells/mm³ in 10–20% of treated individuals.

The level of immune reconstitution in patients with undetectable HIV-1 viral load depends on several factors (e.g., low CD4+ cell count at the initiation of HAART, advanced stage of disease). There is no definitive evidence that age, viral strain/clade, or host genetic factors play a role in these different responses to HAART. The association of CD4 cell nadir with the extent of immune reconstitution in HIV-1-infected individuals suggests that the infection may cause irreparable immune system damage despite HAART. The permanent HIV-1 replication in lymphoid tissues, despite undetectable plasma viral load, has also been proposed as an underlying mechanism for cellular activation and impaired T cell reconstitution. An inadequate production of interleukin (IL)-2
and IL-7 in patients with a long-term HIV-1 infection has been reported to play a role in the lack of CD4 cell increase. Therefore, IL-2 treatment is currently considered a therapeutic option for HIV-1 infected patients with low CD4 cell counts. Furthermore, the baseline immune activation state predicts CD4 cell recovery during viral suppression. In addition, coinfection of several pathogens (e.g., hepatitis C virus) with HIV-1 has been shown to be responsible for enhanced immune activation and may contribute to disease progression and failure of immune reconstitution.

Helicobacter pylori infect the stomach in half of the human population worldwide, causing chronic gastritis, which can lead to peptic ulcer disease, gastric adenocarcinoma, and mucosa-associated lymphoid tissue lymphoma. H. pylori triggers vigorous humoral and cellular immune responses in both systemic and mucosal compartments. In spite of this response, the vast majority of infected hosts are unable to clear the infection, and it persists for decades.

The aims of our study were to assess the prevalence of H. pylori infection among immunologic non-responder HIV-1-infected patients and to assess the possible efficacy of H. pylori eradication on immune reconstitution in this population.

2. Materials and methods

2.1. Study population

Patients were selected from the computerized database of the Neve Or AIDS Center (the biggest AIDS center in Israel). Research staff screened the center’s database and examined the medical and laboratory records to identify HIV-1 patients who had been on HAART for at least 12 months, and who had failed to elevate their CD4 cell count (ΔCD4 <10% after 12 months of HAART) and remained <300 cells/mm³ despite a relatively longstanding (>12 months) plasma HIV-1 suppression (RNA <400 copies/ml). These patients were defined as immunological non-responders. Only patients who met these inclusion criteria were included in the present study. A total of 1284 HIV-infected subjects, 60.7% male and 39.3% female, were found in our center registry. Seven hundred fifty-two of our patients (58.8% male: median age 39 (range 18–74) years; 41.2% female: median age 35 (range 18–64) years) had been treated with HAART for at least 12 months, with regular periodic (every 3 months) determinations of plasma viral load and CD4 cell count. Forty-nine (6.5%) of these patients met the criteria for immunological non-responders. All 49 patients did not have peptic symptoms (asymptomatic H. pylori carriers). At this point, we offered all 26 H. pylori-infected patients a course of triple therapy (a combination of omeprazole 20 mg bid, amoxicillin 1 g bid and clarithromycin 500 mg bid) for 14 consecutive days. Eleven patients (42% of H. pylori-infected HIV-1 patients) agreed and completed H. pylori eradication treatment (group 1). The success of H. pylori eradication was confirmed by a repeat negative UBT, which was done 8 weeks following the termination of the triple therapy. Fifteen patients (58%) refused H. pylori eradication treatment despite our recommendation (group 2). All 26 patients were then followed at our center for at least 24 months, with a periodic (every 3 months) determination of plasma HIV-1 viral load, CD4 and CD8 cell counts. The clinical and laboratory follow-up of the 26 patients (groups 1 and 2) were similar to the routine follow-up of all HIV-1–infected patients at our center (according to the accepted standard of care). Clinical and laboratory data (12 months prior and 24 months following H. pylori diagnosis/eradication) were obtained retrospectively from the center database with the approval of the Kaplan Medical Center Ethics Committee.

2.2. 13C-urea breath test (UBT)

The 13C-urea breath test (UBT) for H. pylori was performed in the central laboratory of Clalit Health Services, Israel. Trained nurses in regional laboratories performed the UBT, and the samples were analyzed by a mass spectrometer (Analytical Precision 2003, UK). The patients were given 75 mg urea labeled with 13C in 200 ml of orange juice, and breath samples were collected before 13C intake (T0) and 30 min later. The cut-off 12C/13C at T0 and T30 time points was 3.5, in accordance with the manufacturer’s instructions.

2.3. Lymphocyte phenotype analysis

Fluorescence activated cell sorting (FACS) analysis (FACScan, Becton Dickinson Immunocytometry Systems, San Jose, CA, USA) was performed on whole heparin-anticoagulated blood within 3 h of collection. A mixture of one to three of the following monoclonal antibodies, conjugated with either fluorescein isothiocyanate (FITC), phycoerythrin (PE), peridinin chlorophyll protein (PerCP) or Cy-Chrome, directed against CD3, CD4 and CD8, was used for the determinations of the relevant subpopulations of T cells.

2.4. Determination of viral load

The HIV plasma viral load was determined by an automated Amplicor polymerase chain reaction and Ampliprlink software (Cobas Amplicor, Roche Diagnostics, Branchberg, NJ, USA). Virologic failure was defined as plasma HIV-1 RNA ≥400 copies/ml in two consecutive determinations after viral suppression (HIV-1 RNA <400 copies/ml).

2.5. Statistical analysis

Results are presented as mean ± standard deviation (SD). The non-parametric Mann–Whitney test and unpaired Student’s t-test

Table 1

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Demographic and immunological characteristics at baseline (time of Helicobacter pylori diagnosis/eradication)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Group 1*</td>
</tr>
<tr>
<td>Number of patients (male/female)</td>
<td>11 (6/5)</td>
</tr>
<tr>
<td>Age (years)</td>
<td>44.5 ± 15.5</td>
</tr>
<tr>
<td>Time from HIV diagnosis, years</td>
<td>5.9 ± 2.4</td>
</tr>
<tr>
<td>Cell counts and percentages</td>
<td></td>
</tr>
<tr>
<td>CD4 cells/mm³</td>
<td>220.3 ± 111.9</td>
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<tr>
<td>CD4%</td>
<td>15.6 ± 8.5</td>
</tr>
<tr>
<td>CD8 cells/mm³</td>
<td>698.3 ± 158.7</td>
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<tr>
<td>CD8%</td>
<td>68.3 ± 12.7</td>
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<tr>
<td>CD4/CD8</td>
<td>0.32 ± 0.11</td>
</tr>
<tr>
<td>Duration of HAART, mean ± SD (years)</td>
<td>2.3 ± 1.1</td>
</tr>
<tr>
<td>HAART**</td>
<td></td>
</tr>
<tr>
<td>AZT + 3TC + NFV</td>
<td>4 (36.4)</td>
</tr>
<tr>
<td>D4T + 3TC + NFV</td>
<td>1 (9.1)</td>
</tr>
<tr>
<td>AZT + 3TC + IDV</td>
<td>0 (0)</td>
</tr>
<tr>
<td>AZT + 3TC + IDV/RTV</td>
<td>2 (18.2)</td>
</tr>
<tr>
<td>D4T + 3TC + IDV/RTV</td>
<td>1 (9.1)</td>
</tr>
<tr>
<td>AZT + 3TC + EVF</td>
<td>2 (18.2)</td>
</tr>
<tr>
<td>AZT + 3TC + NVP</td>
<td>1 (9.1)</td>
</tr>
</tbody>
</table>

Results are mean ± standard deviation or n (%) unless otherwise stated.

* Group 1: 11 patients who underwent successful Helicobacter pylori eradication with triple therapy; group 2: 15 patients who refused H. pylori triple therapy. All patients had H. pylori co-infection as determined by a positive urea breath test and were defined as immunologic non-responders.

** Cell counts and percentages at baseline.

*** HAART (highly active antiretroviral treatment): AZT, zidovudine (300 mg bid); 3TC, lamivudine (150 mg bid); D4T, stavudine (40 mg bid); NFV, nelfinavir (750 mg tid); IDV, indinavir (400 mg tid); IND/RTV, indinavir 400 mg/ritonavir 100 mg bid; EPV, elavirenz (600 mg qd); NVP, nevirapine (200 mg bid).
were used for statistical analyses. Values of \( p \leq 0.05 \) were considered statistically significant.

3. Results

The baseline characteristics of our study population are presented in Table 1. As can be seen in the Table, at the time of \( H. \) pylori diagnosis, the demographic (gender, age, time from HIV-1 diagnosis) and immunological (CD4 and CD8 cell counts and percentages) parameters were similar in both study groups (group 1: 11 patients who underwent successful \( H. \) pylori eradication, as defined by repeat negative UBT, which was done 8 weeks following the termination of \( H. \) pylori triple therapy, and group 2: 15 patients who refused triple therapy for \( H. \) pylori). In addition, the duration and nature of HAART regimens prior to the time of \( H. \) pylori diagnosis were similar in both study groups (Table 1). All patients, in both study groups, revealed HIV-1 suppression (plasma viral load <400 copies/ml) at baseline and during the prior 12 months. In spite of the prolonged (12 months) HIV-1 suppression, these patients (groups 1 and 2) were defined as immunological non-responders since their CD4 cell counts were below 300 cells/mm\(^3\) and/or had not increased by more then 10% per year compared to the levels prior to HAART initiation.

Twenty-four months of follow-up of our study patients clearly demonstrated a significant increase in both CD4 cell counts and percentages in all 11 patients in group 1 following their successful \( H. \) pylori eradication (Figure 1). The CD4 cell counts (and percentages) started to increase at 3 months and peaked at 12–18 months after \( H. \) pylori triple therapy. Thereafter, CD4 cell counts (and percentages) gradually declined. Nevertheless, at the end of the study, 24 months after \( H. \) pylori eradication, the CD4 levels (counts and percentages) were significantly higher compared to the levels during the 12 months prior to \( H. \) pylori eradication (Figure 1). Reciprocal significant changes in CD8 cell counts and percentages were observed in group 1 patients following \( H. \) pylori triple therapy (Figure 2). In contrast, no such changes in either CD4 or CD8 cell counts were observed in group 2 patients (Figures 1 and 2) or in the 23 \( H. \) pylori-negative HIV-1-infected immunological non-responder patients (data not shown).

None of the 11 patients in group 1 demonstrated virological failure (as defined in the Materials and methods) during the 24-month follow-up period. In contrast, four (26.6%) from group 2 and two (8.7%) from the 23 \( H. \) pylori-negative group of patients, revealed virological failure requiring change of their HAART regimen. During the follow-up period there were no significant differences in either CD4 or CD8 cell counts between group 2 patients with virological failure and those without.

**Figure 1.** CD4 cell counts and percentages in the two study groups during the follow-up period. CD4 cell counts and percentages at 12 months (12 to 0) prior and 24 (3 to 24) months following the diagnosis of \( Helicobacter pylori \). Group 1: 11 patients treated with triple therapy resulting in successful \( H. \) pylori eradication; group 2: 15 patients who refused triple therapy. Time 0 indicates the time of \( H. \) pylori diagnosis and eradication. The non-parametric Mann–Whitney test and unpaired Student’s t-test were used to analyze CD4 cell counts and percentages at all time points to prior measurements (e.g., between 3 and 0, 18 and 12 months) and to the levels prior to \( H. \) pylori diagnosis and/or eradication. The absence of star means no statistical significance (\( p \) = NS). **\( p \) < 0.05 between all time points following \( H. \) pylori eradication (3–24 months) and the prior levels (–12 to 0 months). ***\( p \) < 0.0001 compared to previous measurement.
4. Discussion

Our study demonstrates remarkable therapeutic effects of *H. pylori* eradication on immune reconstitution in HIV-1-infected patients with inadequate immunological response despite virological suppression under HAART (immunological non-responders).

Several studies indicate a lower prevalence of *H. pylori* infection in HIV patients as compared to the general population.\(^{14-18}\) In contrast, other studies have reported similar prevalence of *H. pylori* infection in HIV-1-positive and negative populations.\(^{18,15,16,17}\) Moreover, no association between the prevalence of *H. pylori* infection and patient age, sex, risk group, or the type of HAART regimen has been demonstrated,\(^ {18,19}\) though it appears that the prevalence of *H. pylori* infection decreases concomitantly with the decrease in CD4 cell count.\(^{18,20,21}\) In the present study, *H. pylori* infection was found in 26 of 49 (53.1%) HIV-1 immunologic non-responder patients. In agreement with the previous reports,\(^ {22,23}\) all 26 patients had CD4 cell counts of > 100 cells/mm\(^3\). Moreover, *H. pylori*-infected and uninfected subjects (26 and 23 patients, respectively) had similar clinical, immunological (CD4/CD8) and virological (plasma HIV-1 load <400 copies/ml) status. The duration and nature of HAART as well as the demographic background and time from HIV diagnosis were also comparable between *H. pylori*-positive and negative patients.

The mechanisms by which *H. pylori* eradication can boost immune reconstitution in HIV-1-infected patients are not known. As a result of HIV-1 infection, a high proportion of CD4+ T cells are lost, with mucosal tissues being the most severely affected. Indeed, the frequency of HIV-1 infection in gut CD4+ T cells is very high, causing persistent immune activation derived by viral replication leading to further CD4+ cell depletion.\(^{24}\) We propose that *H. pylori* co-infection can further aggravate local and systemic immune activation in patients with chronic HIV-1 infection.

The main cell target during established HIV-1 infection is the CCR5+CD4+ activated T lymphocyte.\(^{25}\) CCR5+CD4+ cells are regularly present in gastric mucosa and their levels increase with inflamed gastric mucosa inflammation.\(^ {26}\) Studies of the local and systemic immune responses against *H. pylori* have led to the concept that *H. pylori* infection stimulates monocytes and macrophages, elicits a predominant Th1 immune response with an increased expression of CCR5 on T cells (the cellular target for HIV-1), and down-regulates cytotoxic T cell responses.\(^ {27-29}\) Moreover, increased expression of the chemokine receptor CCR4, another target of HIV-1, in antral and duodenal activated CD4+ T cells and circulating CD4+CD25\(^{high}\) regulatory T cells have been reported in *H. pylori*-positive individuals.\(^ {30-32}\) Furthermore, *H. pylori* infection may stimulate other HIV-1-infected cells, especially latently infected macrophages, to replicate, as has been shown for other opportunistic infections.\(^ {33-36}\)

It should be noted that some *H. pylori* strains secrete cytotoxin (VacA), which was recently shown to inhibit human CD4+ cell activation and proliferation\(^ {37}\) via blocking of NFAT (nuclear factor
of activated T-cells) activation.\textsuperscript{39} Consequently, VacA might inhibit HIV-1 infection of primary human CD4+ T cells.\textsuperscript{39} Thus, H. pylori eradication may prevent these potentially protective mechanisms. In the present study we did not measure local (gut) or systemic (serum) levels of VacA. Furthermore, the real effects of the latter cytotoxin, if there are any, on the immune system of patients with HIV-1 and H. pylori co-infection, have not yet been determined.

Resting, non-activated CD4+ cells are quite resistant to HIV-1 infection.\textsuperscript{40} Following T cell receptor (TCR) activation, the proliferation and differentiation of CD4+ cells are subsequently driven by sustained cytokine signals. Both TCR and cytokine signals can render resting CD4+ cells highly susceptible to HIV-1 infection.\textsuperscript{41,42} Thus, eradication of H. pylori in HIV-1 patients probably down-regulates CD4+ activation, reducing HIV-1 infection of yet unaffected CD4+ cells. The immune reconstitution seen in our patients following successful H. pylori eradication may be the result, at least in part, of such a mechanism.

In addition, eradication of H. pylori may affect the immune system by other mechanisms, such as improving the absorption and the bioavailability of HAART medications, as was shown previously for delavirdine,\textsuperscript{43} and reducing the translocation of bacterial products that cause immune activation.\textsuperscript{44}

Based on the above studies, which suggest that the H. pylori infection (regarding patient symptoms) might harm the immune system and the rate of immune reconstitution in HIV-1 patients, we offered all our H. pylori-positive HIV-1 patients triple therapy for H. pylori eradication.

Although we suggest that the immune reconstitution observed in group 1 patients was due to successful H. pylori eradication, we cannot rule out other explanations, such as direct immunoregulatory effects of the triple therapy with omeprazole, amoxicillin and clarithromycin\textsuperscript{45} or the elimination of other (undefined) pathogenic infections,\textsuperscript{44} as the basis for the latter effect. Triple therapy of immunological non-responder HIV-1 patients without H. pylori infection for two weeks, which was not done in the present study, may help to clarify this issue.

At the end of the study, 24 months after H. pylori diagnosis and/or eradication, the CD4 and CD8 levels (counts and percentages) in group 1 patients were significantly higher (CD4) and lower (CD8) than the levels observed in the same patients during the 12 months preceding H. pylori diagnosis. However, at the end of the study, the effects were less prominent than at 12–18 months following H. pylori eradication (Figures 1 and 2). A more prolonged follow-up period (>24 months) is needed in order to determine whether the significant difference in immune reconstitution observed between group 1 and group 2 patients is stable or transient. The fact that in 18 months following H. pylori eradication the CD4 cell counts start to decrease with a parallel increase in CD8 cell counts may reflect H. pylori re-infection of group 1 patients (not tested in our study) or it may result from various other factors that affect the immune system in HIV-1-infected patients.

Our study has several limitations. First, the number of patients in both groups was quite small. Second, the patients were not randomly assigned to the two study groups. Third, the study was not blinded, though as outcome parameters were based on laboratory rather then clinical data, a potential bias is less likely. One cannot exclude that the patients in group 2, who refused to take triple therapy for H. pylori eradication, were less adherent to their HAART medications, though the similar clinical and immunological parameters during the 12 months preceding H. pylori diagnosis do not support such an explanation. In addition, as discussed above, a more prolonged follow-up period is needed in order to further evaluate the long-term effects of H. pylori eradication on the immune system of HIV-1-infected patients.

Further studies of larger numbers of HIV-1 patients with and without H. pylori co-infection for a prolonged period of time are needed in order to define the role of H. pylori co-infection and eradication in immune reconstitution of HAART-treated HIV-1-infected patients.

Conflict of interest: No conflict of interest to declare.

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