Regulatory T-Cell Activity But Not Conventional HIV-Specific T-Cell Responses Are Associated With Protection From HIV-1 Infection

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Objective: Two distinct hypotheses have been proposed for T-cell involvement in protection from HIV-1 acquisition. First, HIV-1–specific memory T-cell responses generated on HIV-1 exposure could mount an efficient response to HIV-1 and inhibit the establishment of an infection. Second, a lower level of immune activation could reduce the numbers of activated, HIV-1–susceptible CD4+ T cells, thereby diminishing the likelihood of infection.

Methods: To test these hypotheses, we conducted a prospective study among high-risk heterosexual men and women, and tested peripheral blood samples from individuals who subsequently acquired HIV-1 during follow-up (cases) and from a subset of those who remained HIV-1 uninfected (controls).

Results: We found no difference in HIV-1–specific immune responses between cases and controls, but Treg frequency was higher in controls as compared with cases and was negatively associated with frequency of effector memory CD4+ T cells.

Conclusions: Our findings support the hypothesis that low immune activation assists in protection from HIV-1 infection.

Key Words: regulatory T-lymphocytes, T-lymphocyte, HIV-1, cellular immunity, immunity, immune correlates of protection


INTRODUCTION

As the HIV-1 epidemic continues to spread, with an estimated 2.5 million new infections per year, the development of a prophylactic vaccine remains crucial yet elusive. A challenge to the design of an HIV-1 vaccine is the lack of known natural correlates of protection from infection. A relevant model to identify such correlates is offered by individuals who remain seronegative despite repeated HIV-1 exposures (HIV-1–exposed seronegatives, HESN). Studies of HESN populations have included commercial sex workers, men who have sex with men, injection drug users, infants born from HIV-1–positive mothers and HIV-1 serodiscordant couples, and 2 principal mechanisms involving adaptive immune responses have been proposed to explain protection from HIV-1 acquisition in these populations: the presence of HIV-1–specific immune responses and a general status of immune quiescence.

HIV-1–specific T-cell responses have been examined in HESN cohorts and have been the basis for the design of a number of candidate HIV-1 vaccines. Naturally occurring HIV-1–specific T-cell responses have been observed in uninfected commercial sex workers, homosexual, and heterosexual serodiscordant couples, injection drug users, and neonates born from HIV-1–positive mothers. Such responses are
hypothesized to be generated on repeated HIV-1 exposure and could contribute to inhibition of the establishment of infection.

The term immune quiescence has been used to indicate a lower basal immune activity. Specifically, reduced CD4+ and CD8+ T-cell activation, proinflammatory cytokine secretion, and a quiescent gene expression profile have been observed in HESN as compared with controls, and this has been hypothesized to result in a smaller pool of cells that can support HIV-1 replication.10–15 This reduced immune activation has been proposed to be at least partially due to an increased percentage of regulatory T cells (Tregs),10 a subset of CD4+ T cells with demonstrated suppressive characteristics, with a well-defined role in regulating the immune system under homeostatic conditions and during infection.16

Whether Treg activity or naturally occurring T-cell responses are protective against HIV-1 acquisition has not been well studied in prospective evaluations, largely due to the logistical complexity of obtaining preinfection samples on a large number of subjects, to then be followed for potential HIV-1 acquisition. Within a large, prospective HIV-1 seroincidence study of HESN African men and women, we archived peripheral blood samples for subsequent analyses of natural correlates of immune protection. In the present analysis, we compared T-cell activity in preinfection samples from subjects that became infected with HIV-1 (cases) and HESN that did not acquire HIV-1 throughout the study (controls), thus directly exploring if HIV-specific T-cell responses and/or immune quiescence correlate with protection from HIV acquisition.

**METHODS**

**Experimental Design and Study Participants**

Cryopreserved peripheral blood mononuclear cells (PBMCs) and autologous serum were obtained from individuals participating in the Partners PrEP Study (ClinicalTrials.gov number NCT00557245), a randomized, placebo-controlled clinical trial of daily oral tenofovir disoproxil fumarate (TDF) and (TDF)/emtricitabine (FTC) PrEP among 4747 HIV-1 uninfected couples with HIV-1 serodiscordant partners who had normal renal function, were not infected with hepatitis B virus, and were not pregnant or breastfeeding. They were randomized to once-daily TDF, FTC/CD4+ T cells with demonstrated suppressive characteristics, with a well-defined role in regulating the immune system under homeostatic conditions and during infection.16

Whether Treg activity or naturally occurring T-cell responses are protective against HIV-1 acquisition has not been well studied in prospective evaluations, largely due to the logistical complexity of obtaining preinfection samples on a large number of subjects, to then be followed for potential HIV-1 acquisition. Within a large, prospective HIV-1 seroincidence study of HESN African men and women, we archived peripheral blood samples for subsequent analyses of natural correlates of immune protection. In the present analysis, we compared T-cell activity in preinfection samples from subjects that became infected with HIV-1 (cases) and HESN that did not acquire HIV-1 throughout the study (controls), thus directly exploring if HIV-specific T-cell responses and/or immune quiescence correlate with protection from HIV acquisition.

Phenotype Staining Protocol

PBMCs were thawed and cultured in RPMI (10% fetal bovine serum). Counts and viability were acquired using the TC-20 Automated Cell Counter (Bio-Rad Laboratories, Inc., Hercules, CA). T cells and Tregs were stained with live/dead fixable aqua dead cell stain kit from Molecular Probes (Eugene, OR), followed by cell surface staining with the appropriate cell panel. Surface markers examined for phenotype staining protocol include: CD197 (150503), CD39 (TU66), from BD Biosciences (San Jose, CA); CD3 (OKT3 and HIT3a), CD4 (OKT4), CD8 (SK1) from eBioscience, Inc. Immediately after staining, samples were analyzed using a LSRII automated cell sorter from BD Biosciences (San Jose, CA); CD8 (SK1) from eBioscience, Inc. (San Diego, CA); CD45RA (2H4), CD8-DR (Immu-357), CD3 (UCHT1), and CD14 (RM052) from Beckman Coulter (Marseille, France). Intracellular markers examined include: CTLA-4 (BN13) from BD Biosciences, and FoxP3 (226 A/E7) from eBioscience, Inc. Immediately after staining, samples were analyzed using a LSR II flow cytometer (BD Biosciences) with FlowJo software (Treestar, Inc., Ashland, OR).

Stimulation and Intracellular Cytokine Staining

Stimulations and intracellular staining were performed according to previously published methods.21 Briefly, PBMCs were plated in a 96-well U-bottom plate at 1 × 10^5 cells per well and stimulated with global potential T-cell epitope peptides for HIV-1 Gag, Env, or Tat, each including the 40 most frequent 15-mers among all sequences used to detect CD8+ and CD4+ T-cell responses22–24 in the presence of 10 μg/mL Brefeldin A (Sigma-Aldrich, St. Louis, MO), Golgi stop, and CD107a-APC (BD Biosciences). Peptide diluent (DMSO) served as a negative control and stimulation with 1 μg/mL PMA and 1 μM...
ionomycin (Sigma-Aldrich) served as a positive control. Autologous serum was heat inactivated at 56°C for 30 minutes and 100 µL was added to each well. After a 5-hour incubation at 37°C C/5% CO₂, plates were placed at 4°C overnight. Staining was performed after standard procedures. Flow cytometry analysis was performed using Flowjo software. Live/dead staining was performed using a live/dead fixable aquea dead cell stain kit from Molecular Probes. Antibodies for CD4, CD8, tumor necrosis factor alpha (TNF-α), and interferon gamma (IFN-γ) were purchased through BD Biosciences, CD3 was from Beckman Coulter, and CD14 from Molecular Probes. Samples were collected using a high throughput sample device on a LSRII flow cytometer (BD Biosciences) immediately after staining.

**Statistical Methods**

Cytokine responses to Gag, Env, or Tat peptide pools were defined a priori as CD4⁺ T cells dually expressing IFN-γ and TNF-α, and as CD8⁺ T cells expressing IFN-γ and CD107a after ex vivo stimulation. Sensitivity analyses were also conducted for immune responses defined by a single cytokine. To classify whether a specimen had an HIV-1–specific T-cell response, we compared the proportion of cells positive for the specified cytokine(s) in HIV-1 peptide-stimulated wells to the proportion positive in the DMSO control wells using the statistical model, mixture models for single-cell assays. The magnitude of HIV-1–specific responses was calculated as the percent of cells positive for the cytokine (or combination).

Frequencies of Tregs, T cells and T-cell activation markers were compared by case versus control using a t test. The association between percent Tregs and HIV-1 acquisition was also described by estimating an odds ratio (OR) through logistic regression. Relationships between frequencies of Tregs, T-cell activation and maturation, and magnitude of HIV-specific T-cell responses were estimated using linear regression. Associations between HIV-specific T-cell response magnitude and HIV-1 acquisition were estimated using logistic regression, and between HIV-specific T-cell response status and HIV-1 acquisition using exact logistic regression because of the sometimes small numbers classified as responders. To compare the breadth of peptides recognized in cases versus controls, we restricted to samples responding to at least 1 peptide among those tested for at least 2 peptides and compared the distribution of number of peptides recognized (1, 2, or 3). To account for variation in number of peptides that could be tested for each sample, depending on the cell numbers (2 versus 3), we used a permutation test with 10,000 repetitions of the χ² test. Sensitivity analyses excluding controls that were randomized to PrEP were performed to address the possibility that those controls may have been protected by PrEP rather than by their immune responses, potentially diluting evidence of relationship between immune responses and HIV-1 infection.

Analyses were conducted using SAS 9.3 software (Cary, NC).

**RESULTS**

**Study Population**

All samples were obtained from subjects participating in the Partners PrEP Study. Within this prospective cohort of HESN at high risk of HIV-1 infection as a result of having an HIV-1–infected heterosexual partner, we conducted a nested case-control study to identify correlates of protection among 179 subjects, including 30 who acquired HIV-1 (cases) and 149 who remained HIV-1 uninfected (controls). Specimens from cases were collected a median of 183 days (interquartile range 84–226) before first evidence of HIV-1 infection. Cases and controls were not selected based on their treatment arm, because we previously demonstrated that PrEP does not alter the immunological characteristics analyzed in this study. Cases and controls were comparable for age and sex, and for HIV-1 exposure (Table 1). Assays were run after a preassigned priority scale, depending on sample viability and cell numbers.

**HIV-1–Specific T-Cell Responses in Blood Do Not Protect From HIV-1 Acquisition**

A previously described characteristic of HESN is the presence of systemic HIV-1–specific T-cell responses. We compared frequency, magnitude, and breadth of HIV-specific T-cell responses in cases and controls to determine whether they could protect against HIV-1 acquisition. The frequency of any CD4⁺ T-cell response was 3.4% for cases and 5.4% for controls [OR 0.6, 95% confidence interval (CI): 0 to 5.0, P > 0.99] and any CD8⁺ response was 17.2% for cases and 12.2% for controls (OR 1.5, 95% CI: 0.4 to 4.7, P = 0.64) (Table 2). There was not a statistically significant association between CD4⁺ or CD8⁺ T-cell responses and case/control status when responses were separated by stimulating peptide (ie, Env, Gag, and Tat). Similarly, there was no statistically significant difference when results were stratified by sex (data not shown). Results were comparable in analyses restricting controls to those randomized to placebo. In additional sensitivity analyses of single expression of IFN-γ, TNF-α, or CD107a, no statistically significant difference was observed between cases and controls (data not shown).

Furthermore, we examined the breadth of the responses by evaluating the number of samples responding to 2 or 3 of the peptide pools tested. We did not detect any difference between the 2 groups for CD4⁺ (P > 0.99) or CD8⁺ T cells (P = 0.63).

**TABLE 1. Study Participant Characteristics**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Cases (N = 30)</th>
<th>Controls (N = 149)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female</td>
<td>14 (47)</td>
<td>51 (34)</td>
</tr>
<tr>
<td>Age, yrs</td>
<td>29 (25, 36)</td>
<td>29 (24, 36)</td>
</tr>
<tr>
<td>Number of sex acts, previous mo</td>
<td>6 (3, 9)</td>
<td>4 (3, 8)</td>
</tr>
<tr>
<td>Any unprotected sex, previous mo</td>
<td>14 (48)</td>
<td>72 (49)</td>
</tr>
<tr>
<td>Assigned active PrEP (versus placebo)</td>
<td>14 (16)</td>
<td>73 (76)</td>
</tr>
<tr>
<td>Baseline CD4⁺ T-cell count of HIV-1–infected partner, cells/µL</td>
<td>466 (390, 676)</td>
<td>550 (391, 664)</td>
</tr>
<tr>
<td>Baseline plasma HIV-1 RNA concentration of HIV-1–infected partner, log₀₁₀ copies/mL</td>
<td>4.4 (3.9, 4.7)</td>
<td>4.5 (3.8, 4.9)</td>
</tr>
<tr>
<td>Risk score¹⁰</td>
<td>6 (4, 7)</td>
<td>6 (5, 7)</td>
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10Risk score is the number of sex acts, previous month, number of unprotected sex, previous month, and assigned active PrEP (versus placebo) as defined by the Partners PrEP Study.

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T cells

Finally, we examined the magnitude of the responses by calculating the percent of cells positive for the measured parameters. The magnitude of the responses for CD4+ T cells were comparable for cases and controls; the mean responses (range) for CD4+ T cells were 0.01 and 0.01 on ex vivo stimulation with Env \((P = 0.58)\), and 0.00 and 0.01 with Gag \((P = 0.89)\), 0.01 and 0.01 with Tat \((P = 0.46)\) for controls and cases, respectively (Fig. 1). For CD8+ T cells, the mean magnitudes were 0.03 and 0.02 for Env \((P = 0.86)\), 0.14 and 0.04 for Gag \((P = 0.56)\), and 0.02 and 0.02 for Tat \((P = 0.91)\) for controls and cases, respectively (Fig. 1).

Treg Frequency Correlates With Protection From HIV-1 Infection

Several previous studies have described a state defined as immune quiescence in HESN, characterized by decreased activation of T cells, lower expression of activated genes and a higher frequency of Tregs.25 Thus, we evaluated whether Tregs might affect susceptibility to HIV-1 infection by comparing the frequency of Tregs, identified as CD3+ CD4+ CD25hi CD127dim FoxP3+ cells (Figs. 2A, B), in HIV-1 seroconverting cases and HIV-1 seronegative controls. We detected a significantly higher mean percentage of Tregs in controls, with 3.6% Tregs among CD4+ T cells in controls, versus 3.1% in cases \((P = 0.04)\), corresponding to 35% lower odds of HIV-1 acquisition per 1% higher Tregs \([OR = 0.65, 95\% CI: = (0.43 to 0.98)]\). We observed a similar trend, although not statistically significant, when we defined Tregs as a percentage of CD4+ T cells, with mean values of 5.8% in controls versus 5.1% in cases \([P = 0.08, OR = 0.80, 95\% CI: = (0.62 to 1.03)]\) (Fig. 2C). When we restricted the analysis to samples with a CD4+ or CD8+ HIV-specific T-cell response (as an alternative marker for HIV exposure), we observed a similar pattern but, due to the reduced sample size, the difference between groups was not statistically significant. Similarly, when we limited the analysis to cases from which samples were obtained 6 months or less before HIV acquisition, we observed a similar magnitude of difference, but it was not significant.

To assess Treg functionality, we quantified the frequencies of Tregs expressing the activation markers CD39, CTLA-4, and ICOS, and did not observe any difference in controls and cases. Specifically, CD39 was expressed on 36.8% and 35.2% of Tregs in controls and cases, respectively \((P = 0.75)\), high levels of CTLA-4 were expressed on 43.6% (controls) and 45.1% (cases) of Tregs \((P = 0.45)\), and high levels of ICOS were expressed on 21.1% (controls) and 20.7% (cases) of Tregs \((P = 0.84)\) (Fig. 2D). Finally, as Tregs can be further grouped on the basis of their expression levels of CD45RA and FoxP3 into resting (CD45RA+ FoxP3low) and activated (CD45RA− FoxP3hi) Tregs, we measured the percentages of resting and activated Tregs and did not observe any differences in their percentages in cases as compared with controls (data not shown). Results on Treg frequency and

<table>
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<tr>
<th>Cell Type</th>
<th>Cell Markers</th>
<th>Peptide Pool</th>
<th>Cases</th>
<th>Controls</th>
<th>OR (95% CI)†</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD8+</td>
<td>IFN-γ/CD107a</td>
<td>Gag</td>
<td>5 (17.2) [n = 29]</td>
<td>10 (6.8) [n = 146]</td>
<td>2.8 (0.7 to 10.0)</td>
<td>0.16</td>
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<tr>
<td></td>
<td></td>
<td>Env</td>
<td>3 (11.1) [n = 27]</td>
<td>5 (10.6) [n = 142]</td>
<td>1.1 (0.2 to 4.2)</td>
<td>&gt;0.99</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Tat</td>
<td>2 (7.7) [n = 26]</td>
<td>7 (5.3) [n = 131]</td>
<td>1.5 (0.1 to 8.5)</td>
<td>0.91</td>
</tr>
<tr>
<td>CD4+</td>
<td>IFN-γ/TNF-α</td>
<td>Gag</td>
<td>1 (3.4) [n = 29]</td>
<td>5 (3.4) [n = 146]</td>
<td>1.0 (0.0 to 9.5)</td>
<td>&gt;0.99</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Env</td>
<td>0 (0.0) [n = 27]</td>
<td>4 (2.8) [n = 142]</td>
<td>1.0 (0.0 to 5.9)</td>
<td>0.99</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Tat</td>
<td>0 (0.0) [n = 26]</td>
<td>3 (2.3) [n = 131]</td>
<td>1.3 (0.0 to 8.8)</td>
<td>&gt;0.99</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Any</td>
<td>1 (3.4) [n = 29]</td>
<td>8 (5.4) [n = 147]</td>
<td>0.6 (0.0 to 5.0)</td>
<td>&gt;0.99</td>
</tr>
</tbody>
</table>

†Frequency is reported as N (%) of people responding.
‡OR for association with HIV-1 acquisition.

FIGURE 1. HIV-1-specific T-cell responses are comparable in cases and controls. Magnitude of HIV-specific CD4+ T-cell responses as measured by percentage of cells secreting IFN-γ and TNF-α after stimulation with HIV-1 peptide pools (top). Magnitude of HIV-specific CD8+ T-cell responses as measured by percentage of cells secreting IFN-γ and localization of CD107a on the cell membrane after stimulation with HIV peptide pools (bottom).
activation were comparable in analyses restricting controls to those randomized to placebo (data not shown).

**Tregs Favor the Formation of Central Over Effector Memory CD4+ T Cells**

Given the well-known role of Tregs in controlling the adaptive immune response, we explored the association between Treg frequency and T-cell activation and maturation. As expected, we observed that Treg frequency inversely correlated with CD4+ and CD8+ T-cell activation as measured by coexpression of CD38 and HLA-DR (both $P < 0.0001$, Fig. 3). When we compared CD4+ and CD8+ T-cell activation in controls and cases, we observed that, although the differences were not statistically significant, the frequency of CD38+ HLA-DR+ T cell was lower in controls than in cases. Specifically, the mean percentages of activated CD4+ T cells were 1.4 for controls and 2.1 for cases, and for CD8+ T cells were 1.7 for controls and 2.5 for cases (Table 3).

We next examined the association between Treg frequency and T-cell maturation defined by expression of CD45RA and CCR7 to distinguish naive (CD45RA+, CCR7+), central memory (Tｃｍ, CD45RA−, CCR7+), and effector memory (Tｅｍ, CD45RA−, CCR7−) CD4+ and CD8+ T cells.
cells. Treg frequency directly correlated with the frequency of TCM (P < 0.0001), and inversely with the frequency of TEM CD4+ T cells (P = 0.02) (Fig. 3). Accordingly, the percentage of TCM in controls was higher as compared with cases, whereas the frequency of TEM was lower, although the differences were not statistically significant (Table 3). Given this possible differential modulation of the CD4+ T-cell memory fate, we evaluated the correlation between Treg frequency and the magnitude of HIV-specific CD4+ T-cell responses. We detected a negative correlation between the percentage of Treg and Env (P = 0.007), Gag-specific (P = 0.01) and Tat-specific (P = 0.0025) CD4+ T-cell responses (Fig. 4). In contrast to our findings with CD4+ T cells, Treg frequency did not correlate with TCM or TEM CD8+ T cells (Fig. 3), or with HIV-specific CD8+ T-cell responses (Fig. 4B).

**DISCUSSION**

Although no single factor explaining resistance to HIV-1 infection has been identified, several studies reported unique innate and adaptive immune responses in HESN cohorts. However, nearly all studies on HESN cohorts were retrospective and did not follow subjects in large numbers prospectively to identify whether preinfection immune responses were associated with protection against
subsequent HIV-1 acquisition. Uniquely, the design of our study allowed us to compare immune responses in individuals who maintained an HIV-1–negative status and individuals who became HIV-1 infected (but for whom samples were available for testing at a time previous but close to infection). We focused on 2 possible adaptive immune mechanisms of protection hypothesized to be uniquely found in HESN: the presence of HIV-1–specific T-cell responses, and a general reduced immune activation, known as immune quiescence, both of which could potentially protect individuals from HIV-1 infection.

We first tested whether HIV-1–specific immune responses were associated with protection against HIV-1 infection. We did not find any relationship between HIV-1–specific immune responses and protection against HIV-1 acquisition, either in percent of individuals responding to a particular peptide pool, percent responding to any peptide, numbers of peptides responded to, or magnitude of response. Although CD4+ and CD8+ T-cell responses have been detected in various cohorts of HESN subjects,7,14,18,44,51 their capacity to restrict infection in vivo has not been well studied. Thus, our study, with prospective ascertainment of HIV-1–specific responses, provides a substantial advance in understanding the role of CD4+ and CD8+ responses in HESN. Although we found no association between such responses and protection against HIV-1 acquisition, it is possible that if the responses were of higher magnitude, such as those that could be induced with vaccination, or were located at the site of virus entry, immune protection could be achieved. Furthermore, we focused on IFN-γ/TNF-α “Th1-type responses,” and we did not examine other cytokine secretion, nor CD8+ T-cell cytotoxic molecule synthesis, although vaccine trials using constructs designed to induce CD8+ responses have not demonstrated protective success.52,53

Very recently, using samples collected prospectively as part of the iPrEx Study, Kuebler et al54 demonstrated that particular HIV-1–specific T-cell responses were associated with decreased HIV-1 infection risk. Importantly, an IFN-γ enzyme-linked immunospot assay was used in the iPrEx immunology study, as opposed to our use of intracellular cytokine staining, allowing us to distinguish both CD4 and CD8 T-cell contributions to the HIV-1–specific response, and to develop a stringent positivity call that included dual cytokine positivity. This perhaps accounts for the large discrepancy in reported response rates between the 2 studies: Kuebler et al reported a response rate of over 60% for both cases and controls,54 compared with our CD4+ T-cell response rate of 3.4% for cases and 5.4% for controls and CD8+ T-cell response rate of 17.2% for cases and 12.2% for

**FIGURE 4.** Treg frequency negatively correlates with HIV-specific CD4+, but not CD8+ T-cell responses. Correlation plot and linear regression lines showing the associations between Treg frequency and magnitude of HIV-specific responses to Env (top plot), Gag (central plot), and Tat (bottom plot) mediated by CD4+ (left) and CD8+ (right) T cells.
controls (Table 2). Furthermore, there was no statistically significant difference in responders between cases and controls for the iPrEx immunology study when the cumulative anti–HIV-1 response was considered, similar to our findings, although there were increased responses in controls compared with cases for Gag, Integrase, Vif, and Nef peptide pools considered alone. Similar to our data, Kuebler et al also reported that HIV-1–specific T-cell responses in HESN are generally of low magnitude, with strong responses being infrequent, and so it is unknown how such low-magnitude responses could protect against HIV-1 acquisition, although it remains possible that there are more robust responses at the infection site that were not measured.

Although we found no differences in HIV-1–specific T-cell responses in cases and controls, we did find that the frequency of Tregs was associated with protection from HIV-1 acquisition, with a 1% increase in Tregs corresponding to 35% lower odds of HIV acquisition. As expected, Treg frequency was inversely related to CD4+ and CD8+ T-cell activation. Blunting the immune response could represent an adverse effect of Tregs. Noticeably though, Treg frequency correlated directly with CD4+ TCM and inversely with TEM, which are more likely to constitute a target for HIV-1 given their characteristic ability to traffic to peripheral sites such as the female reproductive tract and gut, potential sites of HIV entry and initial replication. When we compared controls and cases for their frequency of activated CD4+ and CD8+ T cells, and for their frequency of TCM and TEM T cells, we did not observe any difference, possibly due to the limited sample size and the large coefficient of variation between samples. Although not statistically significant, the frequencies were in accordance with the differences in Treg percentage; in fact, lower frequencies of activated T cells and of TEM CD4+ T cells, and higher frequency of TCM were identified in controls as compared with cases. Another likely protective effect that we identified in the study is the inverse correlation between Treg frequency and Env-, Gag- and Tat-specific CD4+ T cells, which could be targeted by HIV if recalled to mucosal sites on viral entry.

Our study presents some limits, such as the lack of data on innate and antibody-mediated immune responses, which have been previously described in HESN cohorts, as well as of responses in the genital and gut mucosa, where the infection initiates in our cohort. Moreover, the number of cases, and thus the statistical power of the study, while substantially larger than previous studies, remained limited. Finally, there was a higher proportion of individuals from the PrEP treatment arm in the control group as compared with the case group. Because PrEP was demonstrated to be highly efficacious in the Partners PrEP Study,17 most cases are naturally from the placebo arm, although the controls came from both the placebo and active arms of the study. However, we recently published a study finding no differences in HIV-1–specific immune responses between PrEP and placebo recipients using these subjects,18 and our sensitivity analysis among the placebo arm was consistent with the overall study findings.

In sum, our results provide new evidence that the maintenance of a low immune activation status mediated by Tregs is beneficial in protecting individuals from HIV-1 infection. The finding is in agreement with a growing literature on the role of immune quiescence in HIV-1 replication and protection,28 although importantly, our unique case-control analysis with preinfection samples rather than an analysis of HESN individuals compared with individuals with low exposure to HIV allowed for us to demonstrate evidence of association between increased Treg frequency and protection from HIV-1 acquisition. In addition, our study found that naturally occurring HIV-specific T cells are not sufficient to protect against HIV acquisition, highlighting that low-magnitude HIV-1–specific T-cell responses observed in HESN may be merely an indicator of exposure rather than a true immune correlate of protection. Together, these findings advance the understanding of the adaptive cellular immunity needed to achieve protection against HIV-1.

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REFERENCES


APPENDIX 1. MEMBERS OF THE PARTNERS PREP STUDY TEAM

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statistician), Robert W. Coombs, Lisa Frenkel, Craig W. Hendrix, Jairam Lingappa, and M. Juliana McElrath.


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