Regulatory T Cell Frequencies Do Not Correlate with Breadth or Magnitude of HIV-1-Specific T Cell Responses

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EDITOR: Despite significant research conducted on regulatory T cells (Tregs) in HIV-1 infection, their role in HIV-1 pathogenesis is not fully elucidated. While there is evidence to support a beneficial effect of Tregs through impact on HIV-1-associated immune activation¹ and more recently viral replication,² other data suggest a negative effect via suppression of critical virus-specific immune responses.^{3,4} Tregs have been shown to suppress HIV-1-specific effector functions *in vitro*^{5,6} and depletion of Tregs in vaccine studies lead to measurable increases in antigen-specific T cell responses in vaccine settings for cancer⁷ and infectious pathogens.⁸ However, the impact of Tregs on *ex vivo* breadth and magnitude of the total HIV-1-specific CD8⁺ T cell response at the epitope level has not been investigated to date.

Comprehensive analyses of HIV-1-specific T cell responses in different cohorts of HIV-1-infected individuals have reported extensive heterogeneity in breadth and magnitude of HIV-1-specific CD8⁺ T cell responses, even in individuals with uniformly undetectable HIV-1 viral load such as HIV “elite controllers.”^{9,10} In this context we hypothesized that elevated Treg frequencies may impact the *ex vivo* breadth and magnitude of the total HIV-1-specific T cell response, a research question unanswered prior to the current study.

To address this question we measured the frequency of CD4⁺CD25⁺FOXP3⁺ regulatory T cells in peripheral blood mononuclear cells (PBMCs) from 44 untreated HIV-1-infected individuals, including 18 HIV-1 elite controllers [HIV-1 viral load (VL) <50 HIV-RNA copies/ml; median CD4 count 849 cells/ μ l, IQR: 686–1209], 21 individuals with chronic untreated HIV-1 infection (median VL 44,500 HIV-RNA copies/ml, IQR: 11,300–69,150; median CD4 count 430 cells/ μ l, IQR: 353–547), and 5 individuals with untreated acute infection (median VL 416,000 HIV-RNA copies/ml, IQR: 228,500–1,613,000; median CD4 count 480 cells/ μ l, IQR: 263–487) [gating scheme Fig. 1a, appropriate CD25 and FOXP3 Fluorescence Minus One (FMO) controls were used to define the CD25⁺FOXP3⁺ population]. We concomitantly screened these study subjects for HIV-1-specific CD8⁺ T cell responses using a panel of 222 HIV-1 optimal epitope peptides tailored to the patients’ HLA-Class-I type in an IFN- γ -ELISpot assay.

For a subset of 17 individuals additional ELISpot data on total T cell responses against the entire expressed HIV-1 genome were generated using a panel of 410 overlapping 15mer peptides spanning all HIV-1 proteins.

As anticipated, breadth (= number of epitopes recognized) and magnitude (= sum of total number of spot-forming cells/million PBMCs) were lowest in individuals with primary HIV-1 infection.⁹ No statistically significant differences in breadth or magnitude were found between elite controllers and chronic progressors (Fig. 1b and c, left panels) and we observed a marked heterogeneity of breadth and magnitude of HIV-1-specific CD8⁺ T cell responses within the HIV-1 elite controllers, despite uniformly undetectable HIV viral load in line with our previously published data.¹⁰

We next correlated the frequency of CD4⁺CD25⁺FOXP3⁺ Tregs to breadth and magnitude of the HIV-1-specific CD8⁺ T cell response in the 44 study subjects and found that neither breadth nor magnitude showed an association with Treg frequencies (Fig. 1b and c; right panels) or absolute Treg numbers ($r=0.19$, $p=0.22$ and $r=0.03$, $p=0.81$, respectively). To account for potential bias toward HLA alleles with many described optimal epitopes, we also generated an optimal epitope recognition score determined by the number of optimal epitopes recognized per individual divided by the number of epitopes described and tested for the respective HLA type. Again, there was no statistically significant difference in optimal epitope recognition scores between the study groups (data not shown) and no correlation with Treg frequency ($r=0.18$, $p=0.24$) or absolute Treg counts ($r=0.10$, $p=0.49$), respectively. Even in elite controllers differences in Treg numbers or frequency did not explain the heterogeneity in HIV-1-specific cellular immune responses.

Based on recent data suggesting differential susceptibility of HIV-1-specific CD8⁺ T cell epitopes restricted by “non-protective” versus “protective” HLA-class I alleles (specifically HLA-B57 and HLA-B27) to Treg suppression,¹¹ we next investigated if associations between Treg frequencies and epitope-specific responses could be identified in our data set after stratification by expression of HLA-B27/B57 ($n=16$) or lack thereof ($n=28$). However, neither breadth nor magnitude

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FIG. 1. (a) Flow cytometry gating strategy for CD4⁺ regulatory T cells (Tregs). Gates are set to include T lymphocytes (CD3⁺) and exclude dead cells (viability marker⁺), monocytes (CD14⁺), and B cells (CD19⁺). Viable CD3⁺CD4⁺ Tregs were defined by the coexpression of CD25 and FOXP3 using appropriate FMO controls. The right panel shows a representative example of CD4⁺ Tregs in an HIV-1-infected elite controller. In all, 18 elite controllers (EC), 21 chronic untreated progressors (CU), and 5 untreated individuals with acute HIV-1 infection (AU) were tested for HIV-1-specific T cell responses to HLA-Class-I-matched optimal epitopes by interferon (IFN)- γ ELISpot assay. Left panels: We determined the (b) breadth (number of CD8 epitopes recognized) and (c) magnitude [total number of spot-forming cells (SFC) per million peripheral blood mononuclear cells (PBMCs)]. Right panels: (b) Breadth and (c) magnitude of the HIV-1 specific CD8⁺ T cell response were correlated to the peripheral CD4⁺ Treg frequencies. Nonparametric tests of significance were performed throughout all analyses, using Kruskal–Wallis testing for intergroup comparisons and the Spearman rank correlation test for bivariate correlations. All data are expressed as medians and p values of 0.05 or less were considered significant. Color images available online at www.liebertonline.com/aid

correlated with Treg frequencies in either group. We also did not observe overall lower Treg frequencies in individuals with HLA-B57 or HLA-B27, possibly mediated by preferential killing of Tregs by HLA-B27/B57-restricted cytotoxic T lymphocytes, as previously suggested.¹¹

Lastly, among the 17 individuals comprehensively screened with 410 overlapping peptides for T cell responses against the entire expressed HIV-1 genome no association between Tregs and breadth and magnitude of the total HIV-1-specific T cell response was found.

Taken together these results suggest that frequencies of regulatory T cells in the peripheral blood do not directly correlate with the breadth and magnitude of HIV-1-specific T cell responses as measured by IFN- γ secretion. Our data assessing CD8⁺ T cell function on the single epitope level by IFN- γ ELISpot confirm and extend previous reports by Owen *et al.*, in which HIV-1-specific T cell immunity was assessed by multiplex cytokine and chemokine analysis in the supernatant of HIV-1-stimulated PBMCs, and similarly no correlation between Treg numbers and HIV-1-induced cytokine production was observed.¹² While cytokine secretion in these two studies did not appear to be impacted by Treg numbers, our data do not rule out that other T cell effector functions such as HIV-1-specific proliferation or cytotoxicity are more closely associated with Treg frequencies. In fact, the differential impact of Treg suppression on proliferation compared to cytokine secretion has been suggested in a recent study.¹¹

While these observations provide a detailed investigation of Tregs and HIV-1-specific T cell responses in peripheral blood, recent data suggest that mucosal and lymphoid tissues may be more enriched in regulatory T cells.^{4,13,14} It is therefore possible that the frequencies of tissue-based Tregs, e.g., in gut-associated or other lymphoid tissue, may have a more direct association with breadth and magnitude of tissue-related HIV-1-specific T cell responses, which warrants further study.

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Author Disclosure Statement

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