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Gag- and Nef- specific responses are associated with increased proportions of regulatory T cells in treated chronic HIV-1 infection

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Introduction

Interventions directly targeting the HIV-1 reservoir to achieve a cure (induction, immune recognition and clearance) are required, and CD4 T cells, mediators of HIV-1-specific response, are central to this. Functional and phenotypic immune profiles associated with slower progression rates have been demonstrated. Early and robust CD4 T-cell responses to Nef and a preserved Gag p24 proliferative response are associated with better disease prognosis. Furthermore, long-term non-progressors have less generalised CD4 T-cell immune activation compared to rapid progressors. This study aims to determine the relationship between virus-specific responses and regulatory T cell (Treg) frequency (Figure 1) in treated chronic HIV-1 infection.

Methods

Peripheral blood mononuclear cells (PBMCs) from ART-treated HIV-1$^+$ individuals were assessed in IFN-γ and IL-2 ELISpot assays (Figure 2 respectively), for their functional responses following stimulation with overlapping pools of Gag and Nef peptides (1,2). Subjects were characterised as being responders to Gag (n=5), Nef (n=3), both Gag and Nef (n=2), or as non-responders (n=7) (Figure 3). Functional responses were then compared to the immunophenotypic profiles using flow cytometry and markers of Tregs (CD4, CD25, CD45RO; Figure 1) (3). Analysis of seronegative donors was also undertaken (n=10). Statistical analysis was performed using the Mann-Whitney U test (Figure 4).

Gating strategy to identify and characterise Treg cells in HIV-1 infection

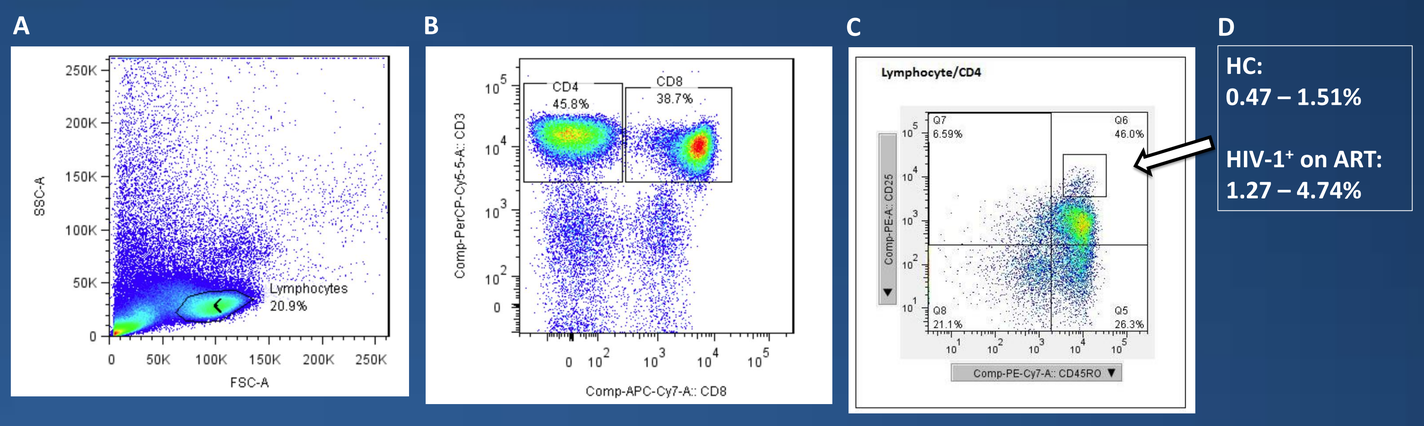

Figure 1. Representative example of flow cytometry staining and gating strategy. Total lymphocytes gated within PBMC (A), expressing CD3 and CD8 (B), and gated CD4 population expressing CD25 and CD45RO (C) depicting the CD25^{high} subset. Box (D) summarises ranges of Treg frequency for both healthy seronegative controls (HC) and HIV$^+$ patients on ART.

Results

All patients had CD4 T-cell counts >350 cells/µl blood and on ART for >12 months with plasma HIV-1 RNA <50 copies/ml. Percentage CD4 Treg subset was significantly higher in the HIV-1$^+$ subjects compared to seronegative donors ($p<0.0001$) (Figure 4). Responders tended to have higher proportions of Tregs, and non-responders lower proportions, albeit higher than observed for seronegative controls (Figure 4). Treg frequencies did not differ between Gag and/or Nef responder groups.

Distinct IFN-γ and IL-2 T-cell responses to overlapping peptide pools of Gag and Nef

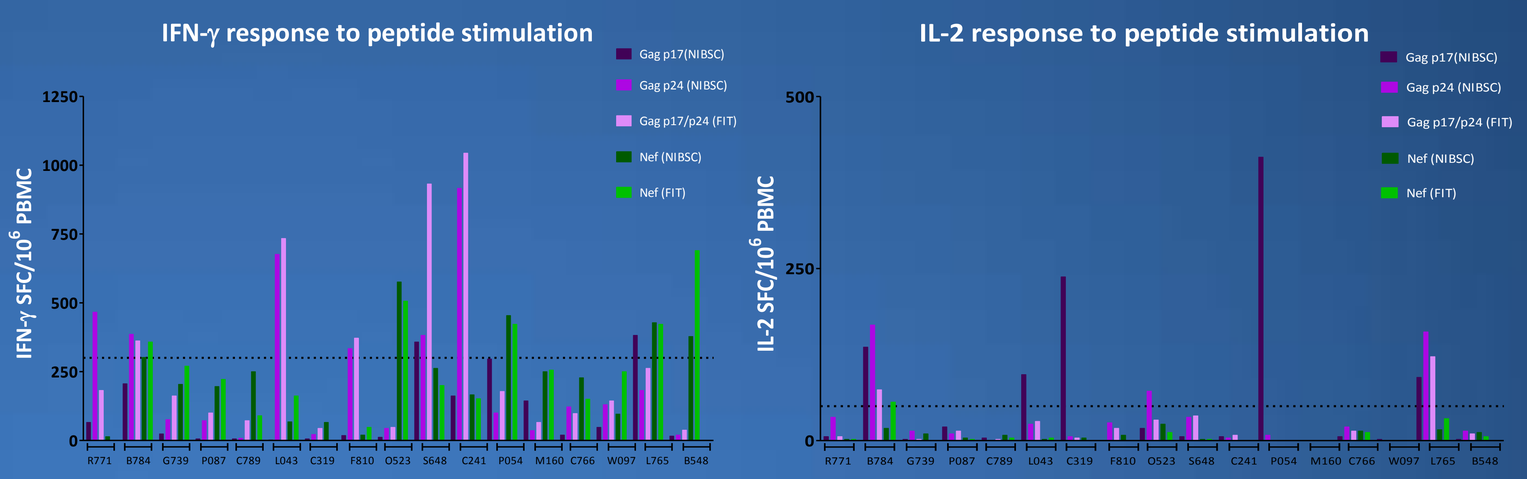

Figure 2. IFN-γ and IL-2 response to overlapping peptide pools of Gag (purple) and Nef (green) from two sources (NIBSC and FIT Biotech; the peptide source and corresponding bar colour is detailed in the key). A cut off of 300 SFC/10^6 PBMC was used for IFNγ to determine which individuals could be defined as responders to Gag, Nef or both, or whether they could be described as 'low responders' for the purpose of these analyses. For IL-2 a cut off of 50 SFC/10^6 PBMC was used to determine positivity. Patient short codes are given on the X axis and n = 17.

Conclusions

Functionally classified responders have increased levels of Tregs during treated infection. This may indicate dysfunction of Treg-mediated suppression. Conversely, elevated Tregs may protect from excessive activation and exhaustion. As Tregs may represent a population enriched for the HIV-1 reservoir in virally suppressed individuals, it is key that the role of Tregs is understood to allow accurate therapeutic targeting in chronically infected individuals. Further in-depth studies, focussing on functional and phenotypic complexity of Tregs during HIV-1 infection and/or therapeutic interventions, are warranted (4, 5).

Distinct responders and related Treg frequencies

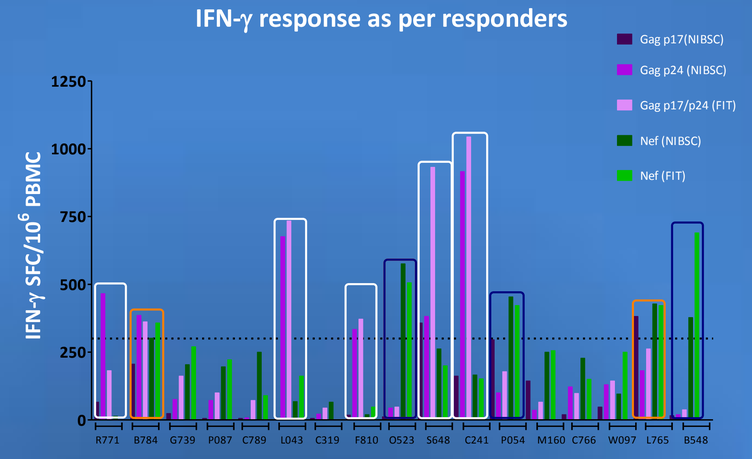

Figure 3. Comparison of Gag, Nef, Gag/Nef and Low-Responders. A cut off of 300 SFC/10^6 PBMC was used to determine which individuals could be defined as responders to Gag, Nef or both, or whether they could be described as 'low responders'

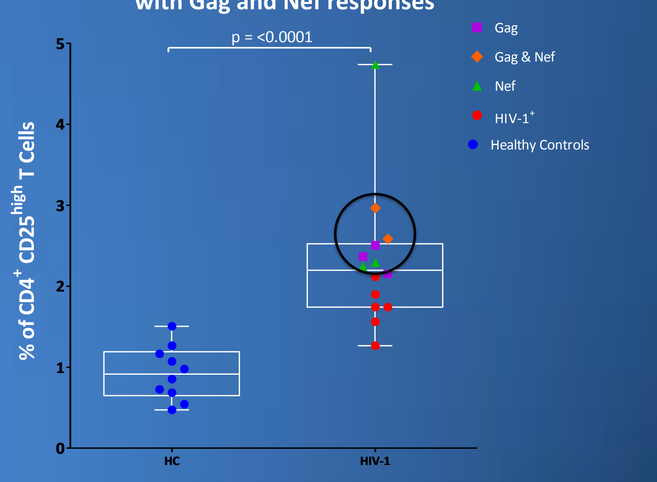

Figure 4. Percentage CD4 Treg subset in the HIV-1$^+$ subjects and healthy controls.

References

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Acknowledgements

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