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Early skewed differentiation and PD-1 expression in CD4$^+$ cells relate to immune dysfunction and viral persistence in individuals living with HIV 1 year post-cART initiation

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Background: Achieving HIV functional cure is a priority. Strategies such as adoptive cell transfer have been assayed, without success yet mainly due to immune dysfunctions observed among individuals.

Aim: To determine the influence of early CD4 T-cell (CD4TC) responses in inflammation levels, viral reservoir, and HIV-specific CD8TC response post-cART

Methods: Samples from 25 HIV$^+$ subjects were collected at diagnosis (baseline sample, BSL) and one year post-cART initiation (post- cART). At BSL, bulk and HIV-specific CD4 phenotype (CD45RO, CCR7, CD95 and PD1 expression) was assessed by flow cytometry after a short stimulation with HIV peptides. Also, proportion of CD4$^+$/HLA-DR$^+$/CD38$^+$ cells was measured. At post-cART, HIV-specific CD8TCs were obtained after 2-week expansion with peptides. Phenotype and antiviral activity (VIA and VITAL assays) were evaluated post-expansion. Plasma CXCL10 (IP-10) was assessed by ELISA. Cell-associated HIV DNA (total and integrated) and unspliced (US) and multiply-spliced (MS) RNA were quantified by real-time PCR. Non-parametric statistics were applied

Results: Early elevated PD-1 on CD4TCs inversely correlated with CD8TCs ability to differentiate post-cART: %CD4$^+$/PD-1^{high} correlated directly with the proportion of stem-cell ($CD8TC_{SCM}$) and central memory ($CD8TC_{CM}$) CD8TC subsets but indirectly with terminal effector ($CD8TC_{TE}$), both in bulk (p=0.018, p=0.0018, p=0.010, respectively) and HIV-specific (p=0.040, p=0.012, p=0.028, respectively) compartments. Similarly, early skewed CD4TCs memory differentiation ($CD4TC_{EM}$/($CD4TC_{EM}$+$CD4TC_{TE}$)) positively correlated with the proportion of $CD8TC_{CM}$ (p=0.0096) and effector memory ($CD8TC_{EM}$, p=0.0031) but inversely with $CD8TC_{TE}$ (p=0.0003) and with the %CD8$^+$/PD-1$^+$ (p=0.023), post-cART. BSL %$CD4TC_{EM,}$ %CD4$^+$/PD-1$^+$ and %$CD4TC_{TE}$/PD1$^+$ correlated directly with CXCL10 post-cART (p=0.037, p=0.0096, p=0.003, respectively). Also, early CD4TC phenotype and activation correlated with viral reservoir: %$CD4TC_{EM}$ inversely with both MS-RNA (p=0.008) and US-RNA (p=0.017); %CD4$^+$/PD-1$^+$ directly with total DNA (p< 0.0001); and %CD4$^+$/CD38$^+$ positively with integrated DNA (p=0.0038) and MS-RNA (p=0.036). Finally, BSL %HIV-specific CD4TCs (p=0.007) directly correlated with CD8-mediated VITAL. In turn, %CD4$^+$/PD-1+ and %CD4$^+$/CD38$^+$/HLA-DR$^+$ correlated inversely with VIA magnitude (p=0.001 and p=0.006, respectively).

Conclusions: Early CD4TC exhaustion, elevated activation and inadequate differentiation seem to be associated with viral persistence, inflammation, as well as with the phenotype and antiviral capacity of HIV-specific CD8TCs that persist one year after cART is initiated. These parameters could serve as predictors of CD8TC function on treated subjects.

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Nef genotype influences immune control in individuals living with HIV-1 who carrying protective alleles

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Background: In-depth understanding of factors leading to immune control in natural course of HIV-1 infection is pivotal in development of prophylactic and therapeutic vaccine. Certain HIV-1 infected individuals carrying protective HLA alleles exhibit durable control of viral replication due to superior CD8+ T cell responses, but extensive heterogeneity exist among these individuals in levels of HIV-1 control. It remains elusive whether and what extent viral factors including Nef-mediated immune evasion function may affect immune control of HIV-1.

Methods: We began by determination of HLA-A and HLA-B downregulation ability of a large panel of 168 Nef clones isolated from chronically, HIV-1 subtype C-infected individuals in South Africa and employed statistical and biochemical approaches to identify Nef genotype associating with HLA downregulation function. Using the resultant Nef genotype data, we explored correlates with HIV- specific T cell responses and plasma viral loads in individuals from South Africa (N=668) and tested the hypothesis again on an independent cohort from Botswana (N=193).

Results: We found that the amino acid polymorphism at Nef position 9 differentially influences HLA-B downregulation function where by the Ser at this position (Ser-9) associated with decreased HLA-B downregulation function; whereas no codon associated with HLA-A downregulation function. This decreased HLA-B downregulation by Nef Ser-9 resulted in increased susceptibility to recognition of a viral antigen by T cell receptor in vitro. Moreover, the protective allele$^+$ individuals infected with viruses harboring Nef Ser-9 exhibited significantly higher HLA-B restricted T cell responses (p< 0.04) and lower viral loads (p< 0.02) compared to those harboring other amino acids at this position . The same observation was corroborated in the independent cohort in Botswana.

Conclusions: Taken together, our results demonstrate that Nef Ser-9 associating with decreased HLA-B downregulation function leads to enhanced immune control in individuals harboring protective HLA alleles, highlighting the importance of Nef-HLA interaction in spontaneous immune control in vivo.

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CD4+ T-cells expressing negative checkpoint receptors are associated with decreased mitochondrial oxidative phosphorylation in chronic HIV

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Background: Chronic HIV is associated with CD4$^+$ and CD8$^+$ T-cells bearing higher frequency of negative checkpoint receptors (NCRs). CD4$^+$ T-cells expressing NCRs contribute to HIV persistence during antiretroviral therapy (ART). We have previously reported that HIV-infected patients had lower mitochondrial Complex I activity compared with HIV-negative controls. We report associations between NCR expression in T-cells and mitochondrial oxidative phosphorylation (OXPHOS) in peripheral blood mononuclear cells (PBMCs) among chronically HIV-infected patients on ART.

Methods: The Hawaii Aging with HIV cohort enrolled patients with documented HIV infection, age≥40 years old, and on stable ART ≥3 months. Multiparametric flow cytometry was performed on cryopreserved PBMCs to quantitate the percentages of CD4$^+$ and CD8$^+$ T- cells expressing exhaustion markers (PD-1/TIM-3/TIGIT). Spearman's correlations were used to identify cross-sectional associations between NCR-expressing T-cells and previously assessed mitochondrial Complex I (NADH dehydrogenase) and IV (cytochrome *c* oxidase) activities in PBMCs.

Results: Of 43 HIV+ patients, median age was 51 years, current CD4 count 518.0 cells/uL, and nadir CD4 count 93.5 cells/uL. Majority (88.4%) were male and 83.7% had undetectable plasma HIV RNA< 50 copies/ml. Four patients (9%) were on zidovudine. Higher CD4 count was associated with higher Complex I (rho=0.33,