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Elevated Caspase-3 Expression and T Cell Activation in Elite Suppressors

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Elite suppressors (ES) represent a unique population of HIV infected persons who control HIV replication below detectable levels without antiretroviral therapy. This group constitutes less than 1% of the HIV-infected population 1, and there have been few comparisons with HIV-uninfected persons and other HIV-infected groups 1–4. Previous research has shown that, regardless of host-mediated viral containment, some ES demonstrated continuous CD4+ T cell loss and increased T cell activation 1. One of the mechanisms that may contribute to T cell loss is activation-induced cell death (AICD) of CD4+ T cells 5. AICD is one of two apoptotic pathways that trigger the activation of caspase-3 6. Caspase-3, a cysteine protease, is a pro-apoptotic marker involved in the signaling cascade of cellular apoptosis, and is activated downstream upon its cleavage by other caspases 7–9. In previous studies involving antigen-stimulation of CD4+ T cells, caspase-3 expression correlated directly with HIV-1 viral load and inversely with CD4 count 6. Increased AICD in untreated HIV-infected individuals has been reported and is the likely mechanism of CD4+ T cell loss in HIV disease 5. This study was undertaken to understand if naturally controlled ES were immunologically different from subjects that contained the virus through effective HAART.

This study was nested within the Women's Interagency HIV Study (WIHS), an ongoing multi-site prospective study of HIV among US women 10. Semiannual visits include

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interview, clinical exam, and collection of biologic specimens. Multi-parameter flow cytometry was utilized to measure intracellular expression of activated caspase-3 and surface expression of T cell activation markers (HLA DR+CD38+) in CD4+ and CD8+ T cells from 3 groups of participants: (i) ES (n=10), women with at least 1.5 years of HIV RNA ≤80 copies/ml in the absence of antiretroviral therapy 1; (ii) successfully treated HIV+ (n=9), women on antiretroviral therapy with suppressed HIV RNA (≤80 copies/mL) and CD4 counts≥350 cells/mL were chosen as a comparator group to study the impact of highly active antiretroviral therapy (HAART) versus natural control in ES; and (iii) HIV-uninfected controls (n=6). Of the 25 study participants, 11 (44%) were African-American and 7 (28%) were Caucasian; the median age was 39 years (interquartile range: 31–45). Study groups did not differ significantly by race ($p>0.05$). HIV-uninfected controls (median age 24 years) were younger, and ES had a significantly lower median CD4+ cell count than successfully treated participants (521 vs. 714 cells/ml, $p=0.03$). The ES and successfully treated controls did not differ by age (median 41.5 vs. 44 years, $p>0.05$).

The cryo-preserved peripheral blood mononuclear cells (PBMCs) were thawed, rested overnight at 37°C in an incubator supplied with 5% CO_2. The next day the cells were stained by first adding LIVE/DEAD® Fixable Aqua stain (Molecular Probes, Inc., Eugene, OR) to identify non-viable cells, followed by monoclonal antibodies: Pacific Blue-conjugated anti-CD3 (BD Pharmingen™, San Jose, CA), PE-Texas Red-conjugated anti-CD4-PE Texas Red (Invitrogen, Carlsbad, CA), and APC-H7-conjugated anti-CD8 (BD Biosciences, San Jose, CA) for T cell subset analysis, and PE-Cy7-conjugated anti-HLA-DR (BD Biosciences) and PerCP-Cy™5.5-conjugated anti-CD38 (BD Pharmingen™) for T cell activation. The cells were permeabilized prior to intracellular staining with FITC-conjugated anti-active Caspase-3 (BD Pharmingen™) for pro-apoptotic expression analysis. The cells were fixed with 2% formaldehyde. Flow cytometry acquisition was performed on a BD LSRII (BD Biosciences) instrument and analyzed by FlowJo (Tree Star, Inc., Ashland, OR). Nonparametric analysis of variance (ANOVA) with Dunn's multiple comparisons was conducted as the sample size was small and data were non-normally distributed.

Our results demonstrated significantly higher caspase-3 expression in ES versus the HIV-uninfected controls in both CD4+ ($p<0.05$) and CD8+ ($p<0.01$) T cells (Fig. 1a). Similarly, the successfully treated HIV+ women had significantly elevated levels of caspase-3 in both CD4+ ($p<0.05$) and CD8+ ($p<0.01$) T cell subsets compared to the HIV-uninfected controls. No significant differences were observed between the two HIV-infected groups.

Both CD4+ and CD8+ T cell activation levels were higher among ES as compared to the uninfected controls. Successfully treated HIV+ women had higher CD8 T cell activation compared to uninfected controls (Fig. 1b). However, this difference did not attain statistical significance ($p>0.05$).

Overall, observations from this study indicate that both ES and successfully treated HIV+ subjects exhibited significantly elevated spontaneous T cell caspase-3 expression compared to HIV-uninfected controls. This increased level of caspase-3 expression may be due to residual HIV replication among both ES and HAART treated subjects. As previously demonstrated using a single copy assay both these groups had equivalent low levels of HIV. 11 In conclusion the increased level of caspase- 3 expression in CD4 T cells of ES coupled with decreased CD4 levels compared to HAART treated subjects suggests that ongoing low level of viral replication may ultimately contribute to immune decline via AICD. Immune restoration in these natural controllers may require antiretroviral therapy or other immune modulatory treatment.

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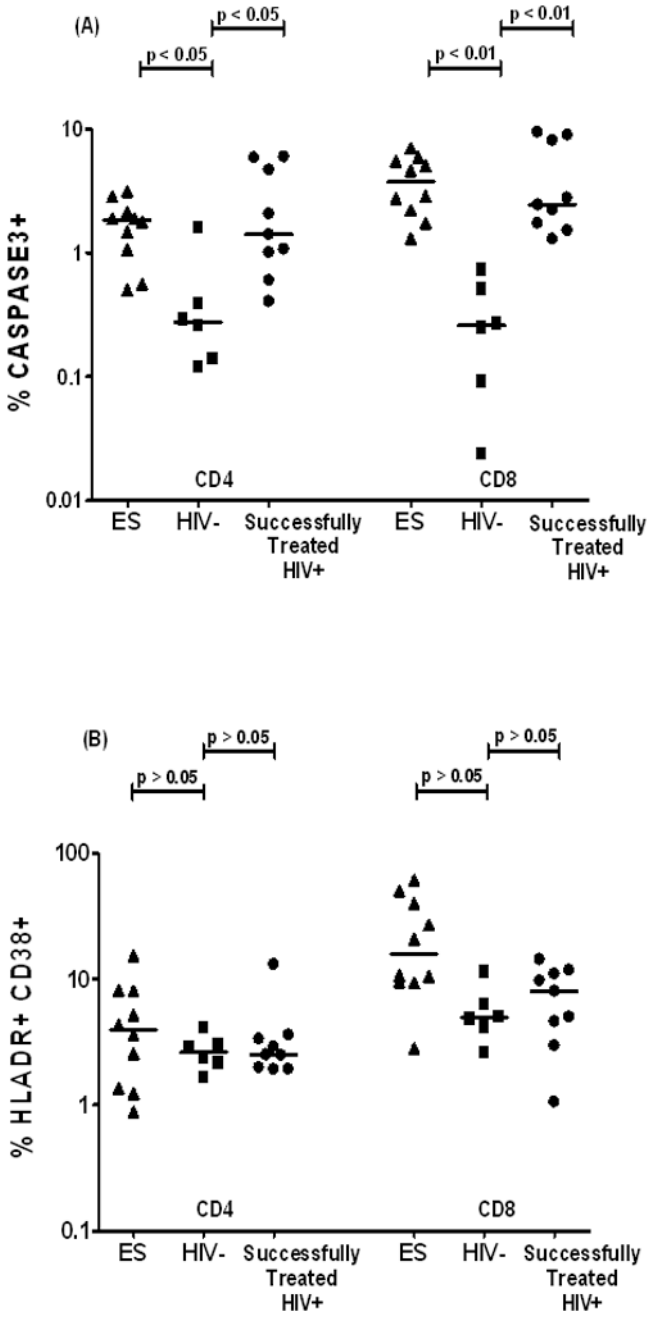

Figure 1. Elevated caspase-3 and T cell activation in Elite suppressors (ES)

Scatter plots exhibit the % expression of spontaneous activated Caspase-3 (A) and HLA DR +CD38+ (B) in CD4+ and CD8+ T cells from PBMCs of HIV-uninfected controls (HIV, n=6), elite suppressors with VL≤80 (ES, n=10) and HIV-infected individuals on optimally suppressive antiretroviral therapy with VL≤80 CD4 ≥350 cells/mL (successfully treated HIV+, n=9).