AIDS RESEARCH AND HUMAN RETROVIRUSES
Volume 30, Number 5, 2014

DOI: 10.1089/aid.2013.0243

Short Communication: CD4 T Cell Declines Occurring During Suppressive Antiretroviral Therapy Reflect Continued Production of Casp8p41

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Abstract

Most patients on suppressive antiretroviral therapy (ART) experience improvements in CD4 T cell count. However, some patients with undetectable viral load continue to lose CD4 T cells for unknown reasons. Casp8p41 is a host-derived protein fragment that is present only in productively infected cells and that causes the death of HIV-infected cells. We questioned whether ongoing $CD4^+$ T cell losses while on suppressive ART were associated with subclinical HIV replication causing production of Casp8p41. We analyzed the association of Casp8p41 content with subsequent CD4 losses in patients on continuous suppressive ART and in patients who discontinued ART after Casp8p41 content was determined, adjusting for age, baseline $CD4^+$ T cell count, and baseline HIV RNA level. Casp8p41 expression in memory $CD4^+$ T cells was measured by intracellular flow cytometry and was correlated with viral load and $CD4^+$ T cell change over time. In patients who stopped therapy after Casp8p41 content was determined, baseline Casp8p41 content did not predict $CD4^+$ T cell change. However, in patients on continuous ART, higher baseline Casp8p41 content was associated with a greater odds of a $CD4^+$ T cell decline at 6 months ($p = 0.01$). Therefore, patients on suppressive ART, who have ongoing production of Casp8p41, have an increased risk of CD4 T cell losses, suggesting that subclinical HIV replication is driving both Casp8p41, which in turn causes a $CD4^+$ T cell decline.

A subset of HIV-infected patients initiating antiretroviral therapy (ART) experiences impaired immune recovery despite virologic suppression, and remains at increased risk of opportunistic infections and death.[1] The reasons for this are unclear, and there are no reliable biomarkers to define this at-risk population. HIV plasma viral load does not explain much of the variability in $CD4^+$ T cell decline among untreated patients.[2,3] Detection of persistent low-level viral replication in treated patients is of uncertain significance, and no consensus exists on how to manage these patients.[4] Although a number of biomarkers related to immune activation as well as innate and adaptive immune cell function have been correlated with HIV disease progression phenotypes, few have been validated, and none is specific to HIV infection.[5]

Casp8p41 is a protein fragment unique to HIV infection that is generated by HIV protease-mediated cleavage of the host protein procaspase 8.[6] Casp8p41 induces HIV^- long terminal repeat (LTR)-dependent transcription of HIV, NF-κB-dependent proinflammatory cytokine production, and mitochondrial-dependent apoptosis through a Bax/Bak-dependent mechanism.[7–9] T cell lines deficient in procaspase

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8 are resistant to HIV-induced cell death, a phenotype that is rescued with reconstitution of wild-type procaspase 8, but not a mutant procaspase 8 that is unable to be cleaved by HIV protease.[10] Some HIV-infected patients who have preserved $CD4^+$ T cell counts despite virologic failure of ART have mutations in the HIV protease gene that selectively impair the ability of HIV protease to generate Casp8p41.[11] These cumulative findings suggest that Casp8p41 is an important mediator of HIV-infected cell death, and may be a significant contributor to HIV disease progression. Indeed, Casp8p41 expression in memory $CD4^+$ T cells has previously been inversely correlated with absolute $CD4^+$ T cell count; in addition, in patients initiating ART, decreases in Casp8p41 expression after initiation of ART were predictive of subsequent increases in $CD4^+$ T cell count.[12]

In this study, we assessed whether virologically suppressed HIV-infected patients had ongoing production of Casp8p41, and if so whether Casp8p41 content in memory $CD4^+$ T cells was associated with an increased risk of subsequent declines in $CD4^+$ T cell number.

Subjects for this study were recruited from the SMART (Strategies for Management of Antiretroviral Therapy) study (ClinicalTrials.gov number NCT00027352), which was a randomized trial comparing continuing ART (viral suppression or VS group) versus ART treatment interruption guided by $CD4^+$ T cell count (drug discontinuation or DC group) in HIV-infected patients.[13] Samples from 220 HIV-infected participants (110 VS and 110 DC) who consented to store cryopreserved peripheral blood mononuclear cells (PBMCs) were obtained for this study. These participants were on ART at baseline. All subjects provided written informed consent to participate in the SMART trial, and all studies were conducted under approval of the appropriate Institutional Review Boards in accordance with all federal regulations.

Casp8p41 expression in memory $CD4^+$ T cells was determined at baseline by flow cytometry as previously validated.[12] T cell subsets were confirmed by fluorescence-minus-one controls. Casp8p41 expression was defined as the percent of live memory ($CD27^+CD45RO^+$ or $CD27^-$) $CD4^+$ T cells positive for intracellular Casp8p41 staining using the naive ($CD27^+CD45RO^-$) $CD8^+$ T cell subset as a negative control (Supplementary Fig. S1; Supplementary Data are available online at www.liebertpub.com/aid). Flow cytometry was performed on an LSRII flow cytometer (Becton Dickinson, Franklin Lakes, NJ), and data were analyzed using Flowjo software (Tree Star, Inc., Ashland, OR).

The association of Casp8p41 content with plasma HIV RNA level and with $CD4^+$ count at entry was studied with the Mann–Whitney test and Spearman rank correlation test, respectively. The association of Casp8p41 content at entry with $CD4^+$ T cell change through 6 months after randomization was studied using linear regression analysis. Casp8p41 content was expressed as a percentage of memory CD4 T cells and patients were grouped into three categories of approximately equal size, zero and above and below the median level of nonzero proportions (<0.012 and ≥ 0.012). For the VS group, a logistic regression analysis was also carried out and the outcome was defined as a $CD4^+$ T cell decline from baseline (versus no change or increase). For this analysis, adjusted odds ratios (ORs) and 95% confidence intervals (CIs) are cited. For the linear regression analyses differences among the three Casp8p41 groups were assessed with an *F*-test and trend was assessed by assigning each participant the average score within the three Casp8p41 groupings (0, 0.006, and 0.066). For the logistic regression analysis, a likelihood ratio test was used to test the difference among the three groups and trend was assessed in the same way as for the linear regression analysis. For the VS group longitudinal mixed models using follow-up $CD4^+$ counts at 1, 2, 4, and 6 months were also used to compare the three categories of Casp8p41. All statistical tests were two sided. A *p* value less than 0.05 was considered statistically significant.

Among the 220 participants, 180 (93 in the DC group and 87 in the VS group) had sufficient PBMCs to be analyzed. The median age was 46 years; 75.6% were men and 48.9% were black. The median (IQR) $CD4^+$ T cell count was 598 (454, 763) cells/mm^3; all were on ART at baseline and 153 participants (85%) had an HIV-RNA ≤ 400 copies/ml (Table 1). The median (IQR) percentage of memory $CD4^+$ T cells positive for Casp8p41 was 0.007 (0.002, 0.020). In 22% of

Table 1. Characteristics of SMART Participants with Casp8p41 Results Measured at Baseline

	DC group (N = *93*)	*VS group* (N = *87*)	*Total* (N = *180*)
Demographics			
Age (median, IQR)	46	45	46 (39, 51)
Gender (% female)	24.7	24.1	24.4
Black (%)	49.5	48.3	48.9
US (%)	100.0	100.0	100.0
$CD4^+$ (cells/mm^3) (median, IQR)	606	597	598 (454, 763)
$CD4^+$ nadir (cells/mm^3) (median, IQR)	139	205	182 (90, 287)
On ART (%)	100.0	100.0	100.0
On PI (%)	60.2	49.4	55.0
HIV-RNA ≤ 400 copies/ml (%)	89.2	80.5	85.0
Prior AIDS-related illnesses (%)	47.3	35.6	41.7
Hepatitis B or C (%)	24.7	18.4	21.7
6 month follow-up visit			
N	92	82	
$CD4^+$ (median, IQR)	437 (325, 585)	590 (464, 769)	
Change in $CD4^+$ (median, IQR)	-158 (-301, 0)	-14 (-77, 95)	
HIV RNA ≤ 400 (%)	43.5	89.0	

DC, drug discontinuation; VS, viral suppression; IQR, interquartile range; ART, antiretroviral therapy; PI, protease inhibitor.

subjects, there was no expression of Casp8p41 detected in memory $CD4^+$ T cells, and the median of nonzero Casp8p41 percentage was 0.012 (0.006, 0.032).

At study entry, Casp8p41 expression was higher in participants with a plasma HIV load ≥ 400 copies/ml (0.015; IQR: 0.006, 0.073) compared to those with a plasma HIV load < 400 copies/ml (0.007; IQR: 0.001, 0.018) ($p = 0.02$). The rank correlation with baseline $CD4^+$ count was -0.01 ($p = 0.88$); this correlation was 0.07 ($p = 0.36$) among the 153 patients with a viral load of 400 copies/ml or lower and -0.28 ($p = 0.16$) among the 27 patients with a viral load > 400 copies/ml.

The median (IQR) change in $CD4^+$ T cell count at 6 months was -14 (-77, 95) cells/mm^3 in the VS group. Conversely, for patients in the DC group, the median change in $CD4^+$ T cell count at 6 months was -158 (-301, 0) cells/mm^3 (Table 1). In the DC group, baseline Casp8p41 expression did not correlate with $CD4^+$ T cell change after 6 months ($p = 0.93$ and $p = 0.76$ for the difference among three Casp8p41 expression groups and for the trend test based on linear regression, respectively).

For patients in the VS group, lower levels of Casp8p41 expression were associated with larger $CD4^+$ T cell changes ($p = 0.04$ for trend). Among those with a Casp8p41 expression of 0.012 or higher there was increased odds of a $CD4^+$ T cell decline at 6 months ($p = 0.01$ for trend, Table 2) after adjusting for age, baseline $CD4^+$ T cell count, and baseline HIV RNA level. Even in those VS participants who had an HIV RNA level ≤ 400 copies/ml at entry, a high Casp8p41 content was associated with an increased risk of CD4 decline ($p = 0.02$), suggesting that residual low-level viral replication allows for HIV protease activity, which in turn produces Casp8p41, which leads to $CD4^+$ T cell loss. The association of Casp8p41 with $CD4^+$ decline was most evident among those with a Casp8p41 content of 0.012 or higher. In an analysis of VS patients with HIV RNA ≤ 400 copies/ml that considered all follow-up $CD4^+$ counts through 6 months, the average changes from baseline for the three Casp8p41 groups after adjustment for age and baseline $CD4^+$ count were $+0.8, +24.0$, and -26.3 cells/mm^3 (2df, $p = 0.21$).

In conclusion, our study indicates that in HIV-infected participants on suppressive ART, persistent high Casp8p41 expression in memory $CD4^+$ T cells is associated with an increased risk of $CD4^+$ T cell loss at 6 months, even after adjusting for baseline viral load. These findings extend our previous reports of the role of Casp8p41 in CD4 T cell depletion in viremic patients and in ART-naive patients initiating ART.[12]

Casp8p41 production results from the degenerate substrate specificity of HIV protease, which cleaves cellular procaspase 8 between amino acids 355 and 356 to generate the novel, cytotoxic fragment.[6] Casp8p41 is not generated by other stimuli of procaspase 8-dependent apoptosis (i.e., death ligands Fas, tumor necrosis factor, or tumor necrosis factor-related apoptosis inducing ligand).[14] Furthermore, Casp8p41 is present only in productively HIV-infected cells, as evidenced by colocalization with intracellular p24 expression.[14] Therefore, our observation that at study entry Casp8p41 expression is higher in participants with plasma HIV load > 400 compared to ≤ 400 copies/ml is consistent with the underlying biology that productive HIV infection drives Casp8p41 expression,[14] and that Casp8p41 expression increases HIV-LTR transcription,[7] and is consistent with our previous reports.[10,12] Detection of persistent Casp8p41 expression even during suppressive ART is also evidence of ongoing low-level productive viral replication in that setting.

CD4 T cell loss in the setting of HIV infection is a multifactorial process, and has been the subject of a number of recent reviews.[15,16] Broadly speaking, CD4 T cell loss in HIV results from either direct viral cytopathicity or indirect effects. Some examples of direct viral cytopathic effects include Casp8p41 production, intracellular accumulation of toxic viral DNA intermediates during abortive infection,[17] and induction of DNA damage response due to viral integrase-initiated DNA breakage.[18] Indirect effects include decreased thymic production of immature T cells, loss of supportive immunologic niches due to tissue fibrosis,[19] microbial translocation,[20] and excessive immune activation.[21] Notably, ART does not fully suppress viral replication,[4] and does not fully normalize microbial translocation and excessive immune activation. Therefore, it is not surprising that Casp8p41 expression is not fully suppressed during effective ART and may have a continued pathologic effect.

One potential limitation of the study was the definition of virologic suppression as an HIV RNA ≤ 400 copies/ml, which was the standard at the time of the SMART trial. It is

Table 2. Association of $CD4^+$ T Cell Change (6 Months—Baseline, Cells/μl) with Casp8p41 Expression at Entry for SMART Viral Suppression Participants

	All VS participants (N = 87)				*VS participants with plasma HIV RNA ≤ 400 copies/ml (N = 70)*			
Casp8p41 content[a]	*No.*	*Mean change (SD)*	*Percent declining*	*Adjusted*[b] *OR (95% CI)*	*No.*	*Mean change (SD)*	*Percent declining*	*Adjusted*[c] *OR (95% CI)*
0	20	36 (172)	44.4	1.00	18	−4 (117)	50.0	1.00
0.001–0.011	33	46 (174)	45.2	1.14 (0.34, 3.78)	27	44 (152)	42.3	0.78 (0.22, 2.82)
0.012+	34	−22 (150)	71.9	3.99 (1.11, 14.4)	25	−38 (162)	73.9	3.32 (0.81, 13.5)
p-value for difference among 3 groups		0.11		0.03		0.15		0.05
p-value for trend		0.04		0.01		0.11		0.02

[a]Percentage of memory CD4 T cells that are Casp8p41 positive by flow cytometry.
[b]Adjusted for age, baseline $CD4^+$, and baseline plasma HIV-RNA level (≤ 400 vs. > 400 copies/ml).
[c]Adjusted for age and baseline $CD4^+$.

possible that modern viral load assays with a lower limit of detection of 20–40 copies/ml may have prognostic benefit in addition to Casp8p41 measurement.

Casp8p41 expression may be a clinically useful biomarker to predict HIV disease progression or continued immunologic decline while on therapy, which therefore warrants further research. Future studies will also need to address whether measurement of Casp8p41 will predict other clinical outcomes, including AIDS, serious non-AIDS conditions, and/or all-cause mortality in both treated and untreated patients.

Acknowledgments

This work was supported by the National Institute of Allergy and Infectious Diseases at the National Institutes of Health (grants NIH awards AI40384, U01AI042170, U01AI068641, and U01AI046362). This publication was also made possible by the Mayo Clinic Center for Translational Science Activities (grant UL1 RR024150) from the National Center for Research Resources (NCRR) at the National Institutes of Health (NIH). The complete list of SMART investigators has been previously published.[13] We would also like to thank all of the SMART participants.

Author Disclosure Statement

No competing financial interests exist.

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