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Aging Cell. Author manuscript; available in PMC 2013 October 01.

Published in final edited form as:
Aging Cell. 2012 October ; 11(5): 916–918. doi:10.1111/j.1474-9726.2012.00856.x.

Expression of *p16^INK4a^* as a biomarker of T-cell aging in HIV-infected patients prior to and during antiretroviral therapy

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Summary

The $p16^{INK4a}$ tumor suppressor gene is a mediator of cellular senescence and has been suggested to be a biomarker of 'molecular' age in several tissues including T-cells. To determine the association of both active and suppressed HIV infection with T-cell aging, T-cell $p16^{INK4a}$ expression was compared between 60 HIV+ suppressed subjects, 23 HIV+ untreated subjects, and 18 contemporaneously collected HIV-negative controls, as well as 148 HIV-negative historical samples. Expression did not correlate with chronologic age in untreated HIV+ patients, consistent with an effect of active HIV replication on $p16^{INK4a}$ expression. In patients on cART with suppressed viral loads, however, $p16^{INK4a}$ levels were similar to uninfected controls and correlated with chronologic age, with a trend toward an inverse correlation with CD4 count. These data show that $p16^{INK4a}$ is a reliable biomarker of T cell aging in HIV+ patients with suppressed viral loads and suggest that poor CD4 cell recovery on cART may be associated with increased T-cell expression of $p16^{INK4a}$, a marker of cellular senescence.

Assessment of the impact of age, chronic HIV-1 infection and persistent immune activation on immune function in HIV+ individuals remains critically important despite effective combination antiretroviral therapy (cART). Immune function and T-cell replicative capacity decline with age (Haynes and Maue, 2009). If active HIV replication (prior to cART) and persistent inflammation and activation while on cART accelerate this decline, then older HIV+ individuals may be at greater risk for the consequences of accelerated immune aging

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Supporting Information:
Appendix S1 Experimental Procedures

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(aka 'immunosenescence'), and younger successfully treated HIV+ individuals may have immune systems more comparable to older uninfected individuals.

Tumor suppressor mechanisms can contribute to aging of the immune system by decreasing replicative capacity in immune cells (Liu and Sharpless, 2009). The $p16^{INK4a}$ tumor suppressor gene induces a permanent growth arrest termed 'cellular senescence' and expression of $p16^{INK4a}$ increases exponentially with age in most tissues in every mammalian species thus far tested (reviewed by Sharpless and DePinho, 2007). Expression of $p16^{INK4a}$ is not an epiphenomenon of aging, but appears to play a causal role in the age-associated replicative decline of several tissues, including T-cells (Liu et al., 2011). Ablation of $\text{p16}^{\text{INK4a}}$-expressing cells in genetically engineered mice delays some age-related phenotypes such as cataracts and sarcopenia (Baker et al., 2011).

Expression of $p16^{INK4a}$ in human peripheral T-cells increases with chronologic age as well as age-modifying factors and interventions (e.g. smoking and sedentary lifestyle) (Liu et al., 2009; Muss et al., 2011), but the effects of HIV infection on this biomarker are unknown. Given that chronic HIV infection may induce some features of immunosenescence (Deeks 2011), we sought to determine the association of active and suppressed HIV replication with T-cell $p16^{INK4a}$ expression in infected patients.

Results and Discussion

To determine the association of untreated HIV infection with $p16^{INK4a}$ levels in T-cells, we compared untreated HIV+ patients to HIV-negative controls (see Appendix S1). As shown in Fig. 1A, there was a significantly higher level of age-adjusted $p16^{INK4a}$ expression in HIV+ patients compared to HIV-negative controls (p=0.003). Because of this marked increase in expression, there was no correlation between chronological age and $p16^{INK4a}$ levels in untreated HIV+ patients (r=0.19 [95% CI −0.24, 0.56], p=0.35). These data suggest that $p16^{INK4a}$ expression in untreated HIV+ patients is driven by HIV replication rather than chronological age. We next compared T-cell expression of $p16^{INK4a}$ in HIV+ patients on cART with suppressed viral loads to expression in HIV-negative controls. There was no significant difference in age-adjusted $p16^{INK4a}$ expression between HIV+ suppressed and HIV-negative individuals (p=0.49) (Fig. 1B). Chronological age and $p16^{INK4a}$ levels were correlated in suppressed HIV+ patients (r=0.29 [95% CI 0.04, 0.51], p=0.02), indicating that control of viral replication reestablishes this relationship. Comparison of the suppressed and untreated HIV+ patients demonstrated a significant difference in age-adjusted $p16^{INK4a}$ levels (p=0.04); however, this difference was only marginally significant after adjusting for smoking and CD4 count (p=0.10), and not significant after adjusting for smoking and CD4/CD8 ratio (p=0.39), which may be a marker of ongoing immune activation.

A multivariate linear regression model was fit with age, smoking and CD4 as predictor variables and $p16^{INK4a}$ as the response variable in HIV+ patients with suppressed viral loads. There was a significant linear association between age and $p16^{INK4a}$ (partial correlation r=0.32 [95% CI 0.07, 0.53], p=0.01), and smoking was associated with a 0.56 (95% CI 0.00, 1.12, p=0.05) increase in $p16^{INK4a}$ expression. Gender and race were not significantly associated with $p16^{INK4a}$ (p>0.2 for either variable) after adjusting for age and smoking, consistent with previous work (Liu et al., 2009). After adjusting for age and smoking, there was a trend toward a negative association between CD4 and $p16^{INK4a}$ expression in treated patients (partial correlation r=−0.21 [95% CI −0.44, 0.05], p=0.10), although this association was weaker than in untreated HIV+ patients (Figure 2).

These data suggest that untreated HIV replication leads to $p16^{INK4a}$ expression that is substantially above age-associated levels in uninfected individuals. Other stimuli have also been reported to induce $p16^{INK4a}$ expression in an age-independent fashion (e.g. tobacco

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and cytotoxic chemotherapy). Given recent work suggesting that $p16^{INK4a}$ expression compromises T-cell replicative capacity (Liu et al., 2011; Lemster et al., 2008), these results are consistent with the possibility that HIV replication is associated with premature T-cell senescence and may explain the observation that active HIV replication is associated with immune dysfunction over and above the effect on CD4 depletion (Valdez et al., 2000). The possible contribution to $p16^{INK4a}$ expression from relative CD8+ population enrichment and activation in untreated HIV+ individuals cannot be excluded. Our data also suggest that effective antiretroviral treatment that suppresses HIV replication results in $p16^{INK4a}$ levels similar to age-adjusted $p16^{INK4a}$ expression levels in uninfected individuals. Our data are consistent with the murine finding that $p16^{INK4a}$ expression leads to reduced T cell replicative capacity with aging (Liu et al., 2011), since $p16^{INK4a}$ expression was marginally associated with lower CD4 counts even when controlling for age in treated patients. Investigating the relationship of this marker of immune aging with markers of immune activation and inflammation that have been shown to be elevated in many HIV+ patients suppressed on antiretroviral therapy may be helpful in understanding HIV-associated immune senescence (Deeks 2011). Ultimately, if validated and correlated with other markers of immune health and function, $p16^{INK4a}$ levels could be used to identify HIV+ patients with premature immune aging, identify risk factors for premature loss of immune function, and to test therapeutic strategies to prevent or reverse HIV induced premature immunosenescence.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We thank Scott Gremillion, Christina Pieringer, and Tyler Hawkins for technical assistance, and Kathleen Tompkins for recruiting HIV+ patients. This work was funded by the UNC CFAR (P30 AI50410) and by R01 AG024379 (to NES).

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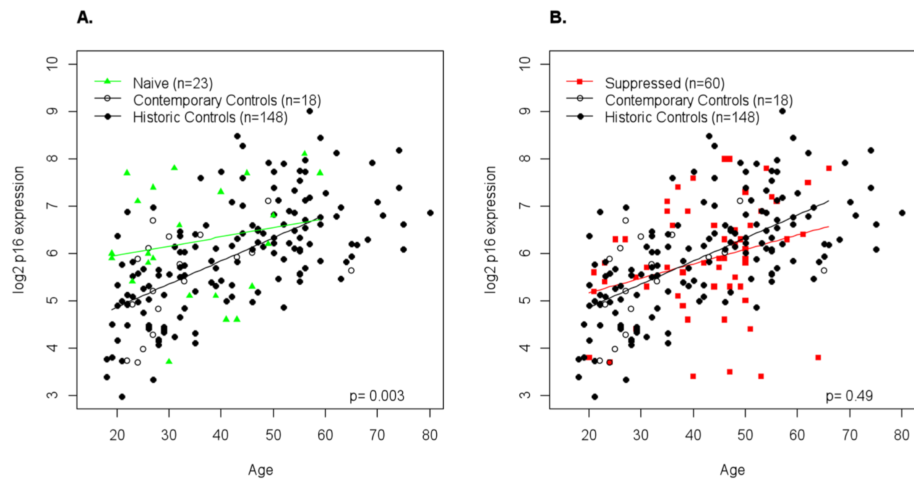

Figure 1.
Comparison of $p16^{INK4a}$ expression levels and age in HIV-negative controls with (A) HIV+ treatment-naïve patients or (B) HIV+ suppressed patients. Solid lines based on group-specific least squares regression.

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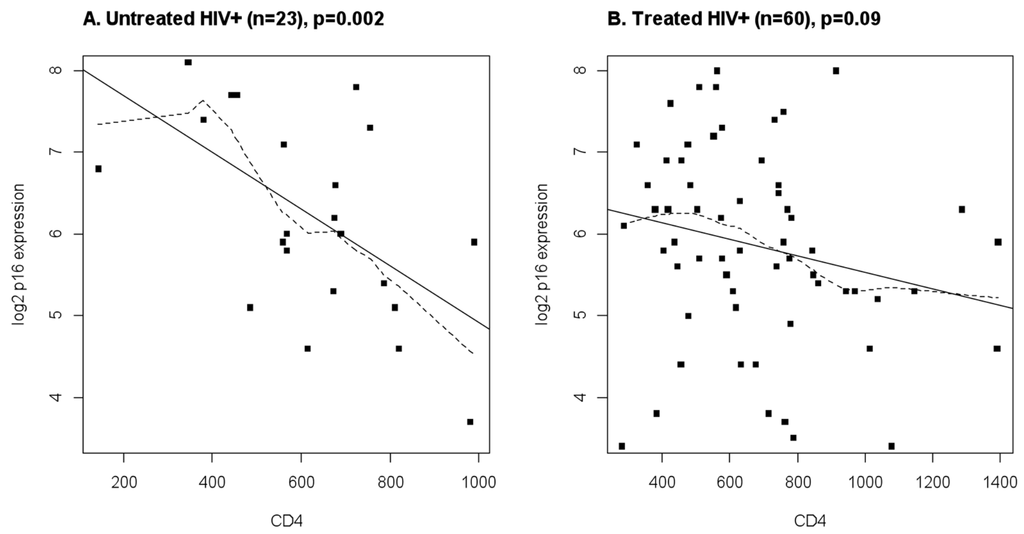

Figure 2.

p16^{INK4a} expression level vs. CD4 count in (A) HIV+ treatment-naïve patients and (B) HIV + suppressed patients. Solid lines as in Figure 1 and dashed lines are from locally weighted polynomial regression (lowess) estimates.