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Prevalence and T cell Phenotype of Slow HIV Disease Progressors with Robust HIV Replication

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To the Editor

Some HIV-infected individuals appear to maintain stable CD4 counts despite high viral replication. Considering that plasma HIV RNA level explains only a small part of the variability of CD4 decline [1], differences in host-mediated immune responses to viral antigen may be an important factor in determining the pace of HIV-1 disease progression.

We hypothesized that some HIV-1-infected individuals better tolerate high viral replication and respond with less immune activation resulting in improved CD4+ T-cell maintenance during untreated HIV-1 infection. To evaluate this we conducted a cross-sectional study within a university-based HIV outpatient clinic to determine the prevalence of slow HIV disease progressors with robust viral replication and determine if these individuals have a *lower* proportion of T-lymphocytes expressing markers of immune activation within differentiation stages, proliferation, activation-induced apoptosis and *greater* expression of regulatory function and naive phenotype markers than fast progressors.

Eligible HIV-1-infected subjects were asymptomatic, antiretroviral-naive, with at least 6 months of follow-up, three or more CD4 measurements and a mean viral load above 20,000 copies/mL. The slope of CD4+T-cell decline was calculated by least squares linear regression method of all available CD4 measurements. Subjects were then qualitatively categorized as 'fast-progressors' if CD4 decline was consistent with rates observed in other untreated cohorts [2,3] or, if less, as 'slow-progressors'. Slow-progressors were randomly selected among eligible patients from the Owen HIV Outpatient Care Center and compared with a convenience sample of recently HIV-diagnosed, asymptomatic, individuals with declining CD4 counts (i.e. fast-progressors) who met the same selection criteria. After appropriate written and informed consent was obtained from all study participants, demographic/ laboratory data and blood samples for peripheral blood mononuclear cells (PBMCs) were collected. No clinical/ laboratory data was collected or analyzed for subjects identified as slow-progressors, but for

whom consent was not obtained and/or were not contacted. Finally, stored PBMC samples from ten HIV uninfected individuals were evaluated as a second comparator group. Statistical tests were performed using the open source statistical package R (version 1.7.0).

Records of all new patients from January 2000 to July 2006 were evaluated (n=2793). Subjects were excluded if they were treatment-experienced (n=1670), lost to follow-up (n=570), had a history of an AIDS-defining illness (n=197) or insufficient lab data (n=86). Of the 467 newly-presenting, antiretroviral-naïve patients, we identified 19 as slow-progressors (4.1% period prevalence). Of these 19 slow-progressors PBMC samples in 6 were compared with samples obtained from 6 fast-progressors. Both slow-progressors and fast-progressors had high presenting CD4 counts (648 vs. 572 cells/mL) and similar durations of HIV infection (3 vs. 2.5 years). Median annual rates of CD4 decline among slow-progressors were -5.6 cells/mm³/year vs. -84.3 cells/mm³/year, despite similar viral loads (4.59 log₁₀ [39,743] copies/mL vs. 4.82 log₁₀ [67,075] copies/mL; P>0.3). Because variability in CD4 measurement can increase at higher counts [4], individual rates of CD4 decline were also adjusted by presenting CD4 counts. Overall, slow-progressors lost less than 1% of their initial CD4 count per year while progressors lost 18% per year.

To determine if increased immune activation, proliferation and apoptosis were associated with more rapid CD4 decline, we determined the frequency of these subsets between slow- and fast-progressors and compared them to HIV-1 uninfected controls. Immune activation was defined as the proportion of CD4+ and CD8+ T-cells expressing CD38, with or without HLA-DR co-expression, and was measured in various maturation phenotypes, including early (CD45RA+CD27+), intermediate (CD45RA-CD27-), and late (CD45RA+CD27-) differentiation steps (Table 1). HIV-1 uninfected controls displayed less immune activation within these CD4+ and CD8+ T-cell subsets (all P<0.01) however, no difference was observed between slow- and fast-progressors. Similarly, CD4+ and CD8+ T-cell proliferation (Ki67+) was also significantly less in HIV-1 uninfected controls, but did not differentiate slow- and fast-progressors. Though the proportion of PHA-stimulated CD4+ and CD8+ (Annexin V+) were similar between all three groups, un-stimulated (CD69-Annexin V+) CD4+ and CD8+ T-cells undergoing apoptosis was significantly lower in HIV-uninfected controls but, again, did not differentiate rates of disease progression.

In this study we did not evaluate markers of thymic output or peripheral expansion to determine whether increased CD4+ production explains the difference in slow- versus fast-disease progression. Rather, we evaluated the proportion of naïve T-cells and regulatory CD4+ T-cell (Table 1) since depletion of regulatory T-cells has been associated with increased immune activation and lower CD4+ T-cell counts during untreated HIV-1 infection [5]. The proportion of naïve (CD45RA+CD27+) CD4+ T-cells were similar for HIV-1 uninfected and HIV-infected with slow- and fast-disease progression (median: 38%, 44% and 50%, respectively). Frequencies of naïve CD8+ T-cells were also similar between HIV-1 uninfected and slow progressions, but there was a trend for lower naïve CD8+ T-cells among fast-progressors and uninfected controls (median: 14% vs. 28%; P=0.05). Differences in the proportion of regulatory CD4+ T-cells varied by how these cells were identified. When defined by FoxP3 expression there was slightly higher frequencies in HIV-1 uninfected controls (P=0.01), but when further sub-grouped into those CD4+FoxP3+ cells with CD25+CD127- there were no significant difference between the three groups.

In this work we sought to address the question as to whether some HIV-infected individuals with robust viral replication respond clinically like sooty mangabey's with SIV infection. First, we selected treatment-naïve individuals with robust viral replication and sufficient follow-up to estimate their rate of CD4+ T-cell decline as a surrogate marker for HIV disease host response. Slow- and fast-progressors were identified from subjects representing opposite poles

of HIV disease progression. This strategy of choosing ‘pure’ cases and controls is appropriate for this type of pilot pathophysiologic study [6]. Second, we attempted to adjust for HIV disease stage and viral load in our selection process by including only asymptomatic individuals with robust viral replication. Although viral load tended to be higher in fast progressors, it is unlikely that an increase of 0.23 log₁₀ copies/mL explains the relatively large difference in rates of CD4 + T-cell loss between these groups. However, using these strict criteria we identified just 19 individuals as slow-progressors from which PBMCs were obtained in only six. This small sample size significantly limited the power of our study and may explain why we did not observe any distinguishing immune phenotypic characteristics between slow- and fast-progressors.

As expected we did observe higher rates of immune activation and spontaneous CD4+ T-cell apoptosis in HIV-infected individuals, regardless of the rate of disease progression, than uninfected controls. And, consistent with a recent report [7], we also observed similar proportions of naive CD4+ T-cells between the three groups. Therefore, if the magnitude of activation-induced apoptosis is similar between slow- and fast-progressors, potentially it is the pace of naive CD4+ T-cell replacement that explains the difference in overall rates of CD4+ T-cell decline.

Slow HIV disease progression despite robust viral replication is an under-recognized disease response in humans that may occur in ~ 4% of untreated individuals. However, in this small study we did not observe any significant differences in the proportions of T-cells that were activated within various memory subsets, proliferating or undergoing apoptosis in slow- versus fast-progressors.

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Table 1**T-cell Phenotypes and Activation-Induced Apoptosis**

Proportions of CD4+ and CD8+ T-cell subsets	HIV-Infected		HIV-Uninfected
	Slow Progressors (n=6)	Fast Progressors (n=6)	Healthy Controls (n=10)
Immune Activation within Phenotype of Maturation			
CD38+ of CD4+CD45RA+CD27+	55 (48–73)	47 (37–54)	46 (25–56)
CD38+ of CD4+CD45RA-CD27–	48 (37–56) ^a	40 (37–42) ^a	12 (10–16)
CD38+ of CD4+CD45RA+CD27–	43 (33–54) ^a	42 (40–41) ^a	24 (23–28)
CD38+ of CD8+CD45RA+CD27+	52 (37–62) ^a	66 (60–70) ^a	14 (11–17)
CD38+ of CD8+CD45RA-CD27–	70 (57–79) ^a	76 (68–78) ^a	16 (14–24)
CD38+ of CD8+CD45RA+CD27–	80 (75–87) ^a	81 (76–86) ^a	26 (20–42)
Proliferating T-cells			
CD4+Ki67+	6 (4–9) ^a	8 (5–11) ^a	2 (1–2)
CD8+Ki67+	5 (5–8) ^a	7 (6–10) ^a	2 (1–2)
Activation-Induced Cell Death Assays			
CD4+AnnexinV+ (unstimulated CD69-)	21 (16–28) ^a	32 (25–39) ^a	14 (6–16)
CD4+AnnexinV+ (stimulated)	55 (45–68)	64 (55–74)	47 (38–55)
Naïve T-cells			
CD4+RA+CD27+	44 (39–45)	50 (41–53)	38 (28–44)
CD8+CD45RA+CD27+	20 (17–28)	14 (9–22) ^b	28 (23–54)
Regulatory T-cells			
CD4+FoxP3+	2 (2–3) ^a	2 (2–3) ^a	3 (3–4)
CD4+CD25+CD127- (of CD4+FoxP3+)	63 (60–68)	61 (58–64)	67 (62–72)

Data are given as medians and 25th and 75th percentiles. Wilcoxon rank sum tests were used for bivariate comparisons of immune subsets. Adjustments were not made for multiple comparisons.

^aP<0.02 for comparison of slow progressors vs. HIV-uninfected and fast progressors vs. HIV-uninfected

^bP=0.05 for comparison of fast progressors vs. HIV-uninfected