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Intensification of a suppressive HAART regimen increases CD4 counts and decreases CD8+ T-cell activation

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Abstract A significant proportion of HIV-1-infected individuals on suppressive HAART regimens do not reconstitute CD4 counts well. If viral reservoirs persist and impact on CD4 reconstitution in a percentage of cases then addition of another antiretroviral agent could further suppress these reservoirs resulting in enhanced CD4 recovery. To evaluate this possibility, we studied the effect of adding abacavir to a chronically suppressive NNRTI containing HAART regimen for 8 patients on their CD4 count and expression of activation markers. Over the first 24 weeks of intensification, CD4 counts increased significantly ($p=0.028$). This increase continued after a year in follow-up with a significant rate of change in CD4 T-cells of 0.959 ± 1.27 per week. In addition, during intensification changes in the percentage of CD38+CD8+ T-cells over time were significantly negatively correlated with changes in CD4 cell number over time above increases predicted without intensification ($r^2=0.716$, $p=0.008$). These data support the possibility that in certain cases where suboptimal CD4 reconstitution occurs that intensification of the regimen can impact immunologic parameters.

Introduction

Treatment guidelines for HIV-1-infected individuals recommend efforts to restore and preserve immune function and suppress viral load in a durable manner. Most practicing physicians use the latter criterion, as a guide in determining whether the therapy used has been successful. However, the CD4 cell recovery on suppressive HAART regimes is variable and frequently does not achieve full reconstitution [1–5].

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Reasons for the incomplete CD4 response include a persistent immunologic defect, drug toxicity, a decrease in thymic output, age, hepatitis C positivity and the persistence of viral reservoirs (e.g. [6–8]).

Over the past decade, numerous studies have demonstrated that viral reservoirs may be present in the background of a suppressed viral burden [9–12]. The effect of these reservoirs on disease progression or the ability to reconstitute the CD4 population is not known. Although information is limited, there are data suggesting individuals with lymph node viral load suppression do better than those with measurable lymph node viral RNA in patients with suppressed plasma viral burdens [9,13]. In studies where infected individuals had their suppressive HAART regimens intensified, the outcome design and intent was not directed at evaluating increases in CD4 counts as a function of intensification. These studies, however, did demonstrate that intensification further decreased viral loads and diminished latent CD4 reservoirs [14,15]. These data as well as other supportive data indicate that ongoing viral replication persists in many cases and the clinical sensitivity of the plasma viral load assays do not fully represent the extent of the viral burden [12,16]. The role these reservoirs have in blunting CD4 responses to HAART, if any, is not known.

In order to evaluate whether intensification of a stable suppressive HAART regimen has an impact on CD4 cell recovery, we performed a pilot study in a cohort of patients who had maintained a stable CD4 count for at least 1 year who had not achieved full CD4 cell reconstitution. For this group of patients, we intensified their NNRTI containing HAART regimen with abacavir and measured changes in CD4 count over time and evaluated whether changes in CD4 count were correlated with changes in CD8 and CD4 T-cell activation.

Materials and methods

Human subjects and study

HIV-1-infected patients followed in the Adult HIV Outpatient clinics at the Jackson Memorial Hospital (JMH)/University of Miami Medical campus were considered for participation in the study if they had stable, plateau CD4 counts between 100 and 400/mm^3 and a non-detectable viral load (<400 copies (c)/ml) for a period of 1 year or more. Patients were included if they were on an NNRTI-HAART containing regimen with background NRTIs consisting of lamivudine and either zidovudine, stavudine or didanosine for a year or more. Ten patients gave informed consent (approved by the Human Institutional Review Board of the University of Miami) to participate. Stable NNRTI containing regimens were maintained and intensified with abacavir at a dose of 300 mg twice daily, and followed closely for side-effects at the Infectious Diseases Research Unit. Participants had blood drawn at time zero, 12 weeks and 24 weeks for immunologic studies. After 24 weeks, the patients were returned to the care of their respective primary care/HIV physicians in the Adult HIV Section. Medical records and interviews were used to extract information on CD4 count, viral load and medical history. One patient experienced pruritus after starting the abacavir, and the abacavir was stopped, and one developed an upper respiratory syndrome during the first week of intensification, not related to the abacavir and chose not to continue in the study. One participant was lost to follow-up after the 24 weeks and return to the outpatient clinic. All other participants were continued on abacavir intensification by their primary care HIV physicians. All individuals were asymptomatic at the time of enrollment. All patients were hepatitis C negative.

CD4 count and viral load measurements

In general, absolute CD4 counts and viral load (VL) measurements (Roche Amplicor Assay) were performed at the JMH/University Diagnostic Pathology Laboratories. The sensitivity of the ultrasensitive assay is 50 c/ml. For individuals with a previously measurable VL the laboratory uses a standard assay (<200 c/ml) to report the VL.

Materials

Fluorescent antibodies, PE-anti-CD4, PE-anti-CD8, FITC-anti-HLADR, APC-anti-CD38, PerCP-anti-CD3 and isotypic controls were obtained from BD PharMingen (San Diego, CA). FACS lysing solution was obtained from Becton-Dickinson (San Jose, CA).

Cells

Whole blood was collected in EDTA containing tubes. Freshly isolated blood was used to determine cell activation.

Determination of activation by flow cytometry

After collection, 50 μl of whole blood was aliquoted into 6 ml FACS tubes and fluorescent antibodies added as a cocktail as determined by experiment for activation (CD3:CD8/4:CD38:HLADR) along with isotypic controls. After vortexing, the tubes were incubated for 10 min on ice followed by addition 450 μl of the 1× lysing solution. Following the manufacturers protocol, cells were kept at room temperature for 10 min and then refrigerated (4 °C) until ready to run on the

Table 1 Patient characteristics

	PID	Gender/ethnicity[a]	HAART[b]	Age	Age WB[c] Pos	CD4 at WB
1	166	M/AA	EFV/ddI/3TC	46	42	11
2	219	F/Hisp	NVP/ZDV/3TC	51	49	73
3	1316	F/Hait	NVP/d4T/3TC	61	58	(231)[d] N/A[e]
4	162	M/Hisp	EFV/ddI/3TC	51	48	8
5	83	M/AA	NVP/ZDV/3TC	76	70	146
6	103	M/Hisp	EFV/ZDV/3TC	38	35	N/A
7	722	M/Hisp	NVP/ZDV/3TC	63	58	31
8	1812	M/AA	EFV/d4T/3TC	67	64	132

[a] M = male; F = female; AA = African-American; Hisp = Hispanic; Hait = Haitian.
[b] EFV = efavirenz; NVP = nevirapine; ZDV = zidovudine; d4T = stavudine; ddI = didanosine; 3TC = epivir.
[c] WB = Western blot.
[d] Lowest CD4 count available at the time of the first visit.
[e] N/A = not available.