

HIV-1 Replication Capacity is an Independent Predictor of Pre-Treatment CD4 Lymphocyte Count

Charles Hicks¹, Joseph Eron², Philip Keiser³, Jason Stout¹, Ian Frank⁴, Sonia Napravnik², Julia Giner¹, Prema Menezes², Chelsea Castellano¹, Jodi Weidler⁵, Tina Korich⁵, and Michael Bates⁵

¹ Duke University Medical Center, Durham, NC, USA; ² University of North Carolina at Chapel Hill, NC, USA; ³ University of Texas Southwestern, Dallas, TX, USA; ⁴ University of Pennsylvania, Philadelphia, PA, USA; ⁵ Virologic, Inc., South San Francisco, CA, USA

Charles Hicks, MD
Box 3360 – Duke University Medical Center
Durham, NC 27710
charles.hicks@duke.edu
ph 919-684-4474; fax 919-681-7494

ABSTRACT (original)

Background: Interactions between host and pathogen determine the natural history of infectious diseases. Characteristics of HIV may impact rates of CD4 lymphocyte decline over time and influence the potential for CD4 regeneration with antiretroviral therapy (ART).

Methods: Patients who initiated HAART and achieved long-term viral suppression (HIV RNA <400 copies/ml) after 12 months were identified from clinical databases at Duke University Medical Center, the University of North Carolina, and the University of Texas-Southwestern. Pre-treatment baseline (BL) stored serum samples collected <3 months prior to HAART initiation were analyzed for the presence of phenotypic resistance and HIV replication capacity (RC) using a single cycle pseudo-typed virus construct (Virologic modified PhenoSense assay). Multiple linear regression (SAS v8.2) was used to examine relationships between demographics, viral replication, and CD4 counts.

Results: The study population consisted of 109 ARV-naïve patients initiating antiretroviral therapy- 71 males, 37 females (gender not recorded for one patient), 66 African-Americans, 28 whites, 12 Hispanics, 3 other. Mean age was 40 years [range 19 to 69]. A variety of HAART regimens were used: NNRTI regimens 56%, PI regimens 37%, triple NRTI regimens 7%. Median BL CD4 count was 186 cells/mm³ [range 3 to 1066]; median BL VL was 75,729 copies RNA/ml [range 400 to >750,000]. All patients achieved suppression of HIV RNA to below 400 copies RNA/ml after 12 months of therapy. BL RC and viral load were independently associated with a lower BL CD4 count. In a model adjusting for both variables, for every increase of 1log₁₀ in HIV RNA viral load, BL CD4 tended on average to be 97 cells/mm³ lower (p =0.001), and for every increase of 1 unit (percentage) in the RC, BL CD4 count tended to be 1.13 cells/mm³ lower (p=0.021).

Conclusions: These data suggest that more advanced HIV is associated with both the quantity of viral replication and the fitness of the virus, as measured by the RC assay. RC appears to measure an intrinsic viral characteristic influencing HIV-1 disease progression independently of the magnitude of viral load.

BACKGROUND

- The commercially available phenotypic resistance assay (Phenosense) performed by ViroLogic, Inc. [S. San Francisco, CA] includes measurement of a viral characteristic that has been termed replication capacity (RC)
- A pseudotyped virus containing portions of the gag-pol region of the HIV genome from patient samples is constructed and generation of relative light units (RLUs) induced by luciferase expression is measured in a single cycle of virus replication.
- This patient-derived RLU value is comparing to that measured from a control virus (NL4-3), and a ratio of test virus RLUs to control RLUs is obtained. This measurement is termed RC.
- Previous work has suggested a relationship between the RC of an infecting strain of HIV and the subsequent CD4 count at viral set-point prior to initiation of antiretroviral therapy.
 - A study of 191 patients with acute or recent HIV infection found that an RC ≤43% was significantly associated with higher baseline CD4 counts independent of drug resistance and viral load (Barbour et al. *J Infect Dis* 2004;190:251-6).

METHODS

- Patients who initiated HAART and achieved long-term viral suppression (HIV RNA <400 copies/ml) after 12 months were identified from clinical databases at Duke University Medical Center, University of North Carolina, University of Texas-Southwestern, and University of Pennsylvania
- Pre-treatment serum/plasma samples that had been frozen were assayed to measure RC.

Inclusion criteria: Adult patient, age ≥18 years; documented HIV infection; first time initiation of highly active antiretroviral therapy (at least a 3 drug regimen); ≥12 months of follow-up after HAART initiation; ≥3 documented viral load measurements within the first 12 months of HAART; HIV-RNA <400 copies/ml at month 12 of HAART (window 12-15 months for final measurement); availability of stored serum sample collected ≤3 months prior to HAART initiation

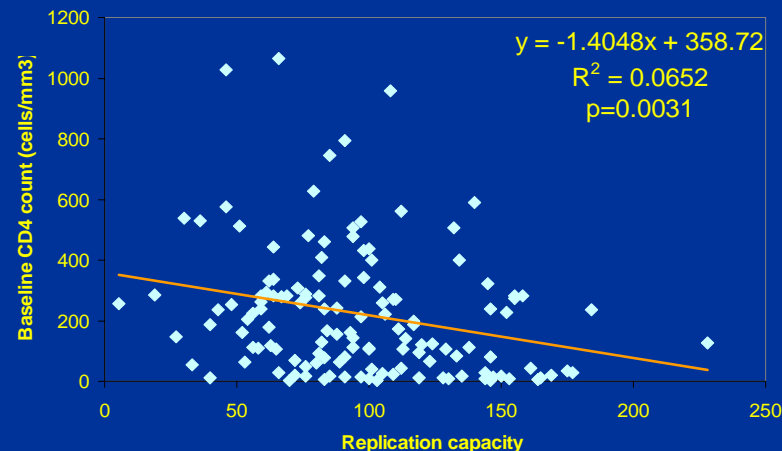
Exclusion criteria: >1 week of prior therapy with any antiretroviral agent; CDC Clinical Category C diagnosis at the time of HAART initiation (active within 4 weeks of initiation of HAART; ≥3 HIV-RNA measurements >400 copies/ml ("blips") in the first 12 months of therapy once undetectable viral load has been achieved; change (other than for toxicity management), interruption, or discontinuation of the initial HAART regimen within the first 12 months of treatment

RESULTS

Table 1. Subject Demographics (N=132)

| Characteristic | N (%) |
|---------------------------|------------------|
| Gender | |
| Female | 41 (31%) |
| Male | 91 (69%) |
| Race/Ethnicity | |
| African American | 80 (61%) |
| Caucasian | 34 (26%) |
| Hispanic | 15 (11%) |
| Other | 2 (2%) |
| Coinfection | |
| Hepatitis B | 20 (15%) |
| Hepatitis C | 16 (12%) |
| Antiretroviral therapy | |
| NRTI only | 9 (7%) |
| NRTI + NNRTI | 69 (52%) |
| NRTI + PI | 49 (37%) |
| NNRTI + PI | 2 (2%) |
| NRTI + NNRTI + PI | 3 (2%) |
| Mean age (range) | 39 (19-69) |
| Mean baseline CD4 (range) | 223 (3-1066) |
| CD4 <200 | 69 (53%) |
| CD4 201-350 | 38 (29%) |
| CD4 ≥350 | 24 (18%) |
| Mean log HIV RNA (range) | 4.90 (3.12-6.14) |

Figure 1. Subjects' baseline CD4 counts prior to antiretroviral therapy vs. HIV replication capacity.



BL RC and viral load were independently associated with a lower BL CD4 count. In a model adjusting for both variables, for every increase of 1log₁₀ in HIV RNA viral load, BL CD4 tended on average to be 151 cells/mm³ lower (p =0.001), and for every increase of 1 unit (percentage) in the RC, BL CD4 count tended to be 0.88 cells/mm³ lower (p=0.0498).

CONCLUSIONS

These data suggest that more advanced HIV as determined by the CD4 lymphocyte count is associated with both the quantity of viral replication and the fitness of the virus, as measured by the RC assay.

RC appears to measure an intrinsic viral characteristic influencing HIV-1 disease progression independent of the magnitude of viral load.

Acknowledgements

The authors gratefully acknowledge the following persons:

- The patients whose samples were used in this study
- Janet Mueller and Stuart Carr for research support
- Chris Petropoulos, Eoin Coakley, and Neil Parkin for helpful suggestions and interpretation of the results
- Funding from NIH – NIAID 1K24 AI01608-011
- Support from Virologic, Inc. S. San Francisco, CA