AIDS RESEARCH AND HUMAN RETROVIRUSES
Volume 21, Number 4, 2005, pp. 285–291

Short Communication

Viral Dynamics and CD4$^+$ T Cell Counts in Subtype C Human Immunodeficiency Virus Type 1-Infected Individuals from Southern Africa

CLIVE M. GRAY,[1] CAROLYN WILLIAMSON,[2] HELBA BREDELL,[3] ADRIAN PUREN,[1] XIAOHUA XIA,[3] RUBEN FILTER,[3] LYNN ZIJENAH,[4] HUYEN CAO,[5] LYNN MORRIS,[1] EFTHYIA VARDAS,[6] MARK COLVIN,[6] GLENDA GRAY,[7] JAMES McINTYRE,[7] ROSEMARY MUSONDA,[8] SUSAN ALLEN,[8] DAVID KATZENSTEIN,[9] MIKE MBIZO,[4] NEWTON KUMWENDA,[10] TAHA TAHA,[10] SALIM ABDOOL KARIM,[6] JORGE FLORES,[11] and HAYNES W. SHEPPARD[5]

ABSTRACT

Defining viral dynamics in natural infection is prognostic of disease progression and could prove to be important for vaccine trial design as viremia may be a likely secondary end point in phase III HIV efficacy trials. There are limited data available on the early course of plasma viral load in subtype C HIV-1 infection in Africa. Plasma viral load and CD4$^+$ T cell counts were monitored in 51 recently infected subjects for 9 months. Individuals were recruited from four southern African countries: Zambia, Malawi, Zimbabwe, and South Africa and the median estimated time from seroconversion was 8.9 months (interquartile range, 5.7–14 months). All were infected with subtype C HIV-1 and median viral loads, measured using branched DNA, ranged from 3.82–4.02 $\log_{10}$ RNA copies/ml from 2–24 months after seroconversion. Viral loads significantly correlated with CD4$^+$ cell counts ($r = -0.5$, $p < 0.0001$; range, 376–364 cells/mm^3) and mathematical modeling defined a median set point of 4.08 $\log_{10}$ (12 143 RNA copies/ml), which was attained approximately 17 months after seroconversion. Comparative measurements using three different viral load platforms (bDNA, Amplicor, and NucliSens) confirmed that viremia in subtype C HIV-1-infected individuals within the first 2 years of infection did not significantly differ from that found in early subtype B infection. In conclusion, the course of plasma viremia, as described in this study, will allow a useful baseline comparator for understanding disease progression in an African setting and may be useful in the design of HIV-1 vaccine trials in southern Africa.

[1]National Institute for Communicable Diseases, Johannesburg, South Africa.
[2]Institute of Infectious Diseases and Molecular Medicine, University of Cape Town, South Africa.
[3]Department of Electrical Engineering, Pretoria University, Pretoria, South Africa.
[4]Department of Immunology, University of Zimbabwe, Harase, Zimbabwe.
[5]Department of Health Services, Richmond, California.
[6]HIV Vaccine and Prevention Trials Unit, MRC, Durban, South Africa.
[7]Perinatal HIV Research Unit, University of the Witwatersrand, Johannesburg, South Africa.
[8]Zambia-UAB HIV Research Project, Lusaka, Zambia.
[9]Center for AIDS Research, Stanford University Medical Center, Stanford, California.
[10]Johns Hopkins Research Project, Blantyre, Malawi.
[11]Division of AIDS, National Institutes of Health, Washington DC.

To gauge the effectiveness of vaccines and antiretroviral therapies (ART) in altering the natural history of HIV-1 infection, it is necessary to understand levels and dynamics of viremia in the absence of vaccination or ART. Several studies have evaluated the natural course of HIV-1 viremia in adults, where it was identified that viremia increased during the early stages of primary infection,[1,2] and declined to an equilibrium or set point.[1,3,4] Some investigations have argued against attaining a set point and that slopes in viremia[5–10] and $CD4^+$ T cell counts[7] over time of infection correlate with AIDS or AIDS-free survival. Natural history studies have primarily been undertaken in developed countries in predominantly male cohorts. Whether these scenarios apply in an African setting is unclear and there are currently few data from cohorts in Africa that describe the course of HIV-1 viremia from known dates of infection. Adult studies in Africa have focused on prospective analyses of CRF02-A/G HIV-1 or HIV-2 viremia,[11,12] subtype A and D HIV-1-infected Kenyan women and men,[13] and cross-sectional studies in female sex workers.[14] A study of female sex workers in South Africa revealed low viral loads in a small cohort[15] and higher viremia in those dually infected with two phylogenetically distinct subtype C strains. Prevalent levels of HIV-1 subtype C viremia have been described in Ethiopian adults[16] and in children from Malawi, where high plasma viral load correlates with mortality.[17] With emerging clinical HIV-1 drug and vaccine trials in southern Africa,[18] knowledge of HIV-1 subtype C viremia provides valuable data for comparative purposes.

In this article we describe the course of viral loads and $CD4^+$ cell counts and the use of mathematical modeling to predict the time taken to reach relatively stable viremia. Sixty-five individuals were recruited into the HIVNET 028 study, a four-country study to investigate the natural history of subtype C HIV-1 infection in Zimbabwe, Malawi, Zambia, and South Africa.* Fourteen participants were excluded from the analysis because of indeterminate times of seroconversion. The median interval time between last antibody negative and first antibody positive was 8.9 months with an interquartile range of 5.7–14 months (Table 1). Participants were monitored at 2, 4, 7, and 9 months after enrollment. Fifty-eight HIV-1-seronegative individuals were recruited as controls. Viral loads were assayed by branched DNA (bDNA) (version 3.0; Bayer, Emeryville, CA), with a limit of detection of 50 RNA copies/ml. Blinded replicate testing of samples was performed, using the reverse transcriptase-polymerase chain reaction (RT-PCR; Amplicor HIV-1 Monitor test version 1.5; Roche Diagnostic Systems, Branchburg, NJ) according to the standard assay (detection limit of 400 copies/ml) or the more sensitive assay (limit of detection, 20 copies/ml) as well as the NucliSens assay (Organon Teknika [Durham, NC], with a detection limit of 125 copies/ml). All samples were shipped within 3 hr of blood draw and processed and stored at −70°C within 24 hr.

The distribution of participants between southern African countries is shown in Table 1, where most participants (42 of 51) were women. Confirmation that the infecting virus was subtype C was shown by population-based sequencing in *gag* (Fig. 1A). Comparisons of $\log_{10}$ RNA copies per milliliter in all participants at enrollment showed no significant differences between countries and were therefore analyzed as a single cohort. Median viral loads between 2 and 6 months was 4.01 (3.48–4.53, IQR); between 7 and 12 months it was 3.82 (3.43–4.4, IQR); between 13 and 18 months it was 4.02 (3.46–4.38, IQR); and at >18 months it was 3.84 (3.29–4.36, IQR). The overall median viral load of 4.0 $\log_{10}$ copies/ml was similar to other subtype C cohorts monitored in Ethiopia[16] and was also within the range of viremia measured in CRF02-infected female sex workers without a sexually transmitted disease in West Africa.[14]

The median $CD4^+$ cell counts measured, using the FACSCount platform (BD Biosciences, Franklin Lakes, NJ), between 7 and 12 months after estimated seroconversion was 376 cells/mm^3 (250–522, IQR); between 13 and 18 months it was 382 cells/mm^3 (255–556, IQR) and at >18 months it was 364 cells/mm^3 (274–511, IQR). $CD4^+$ cell counts from HIV-1-seronegative individuals ($n = 58$), some of whom were recruited at the clinical sites, were used as a reference range, with a median of 625 cells/mm^3 (523–783, IQR). When $CD4^+$ cell counts from the HIV-1-infected cohort were stratified, low $CD4^+$ cell counts (<200 cells/mm^3) were significantly grouped with high viremia and high $CD4^+$ cell counts (600+ cells/mm^3) were significantly grouped with low viremia (Fig. 1B). There was a significant inverse correlation with viral loads ($r = -0.5$,

*Local institutional review board informed consent was given for this study, and the human experimentation guidelines of the U.S. Department of Health and Human Services were followed in the conduct of this clinical research.

Table 1. Details of Participants at Enrollment and Initial Viral Load Measurements

	Malawi	*Zimbabwe*	*Zambia*	*South Africa*	*Total*
n	6	10	16	19	51
Gender	5M:1F	F	4M:12F	F	9M:42F
Median age (years)	33	25	31	25	28
Median interval (months) from seroconversion to first viral load measurement (interquartile range)	8.4 (6.8–9.4)	4.8 (4.4–5.7)	14.6 (13.6–15.3)	7.1 (5.5–12.5)	8.9 (5.7–14)
Median $\log_{10}$ viral load copies/ml at enrollment	4.03	3.61	4.07	3.58	3.97
Geometric mean viral load copies/ml at enrollment	7565	9154	10241	5718	9262

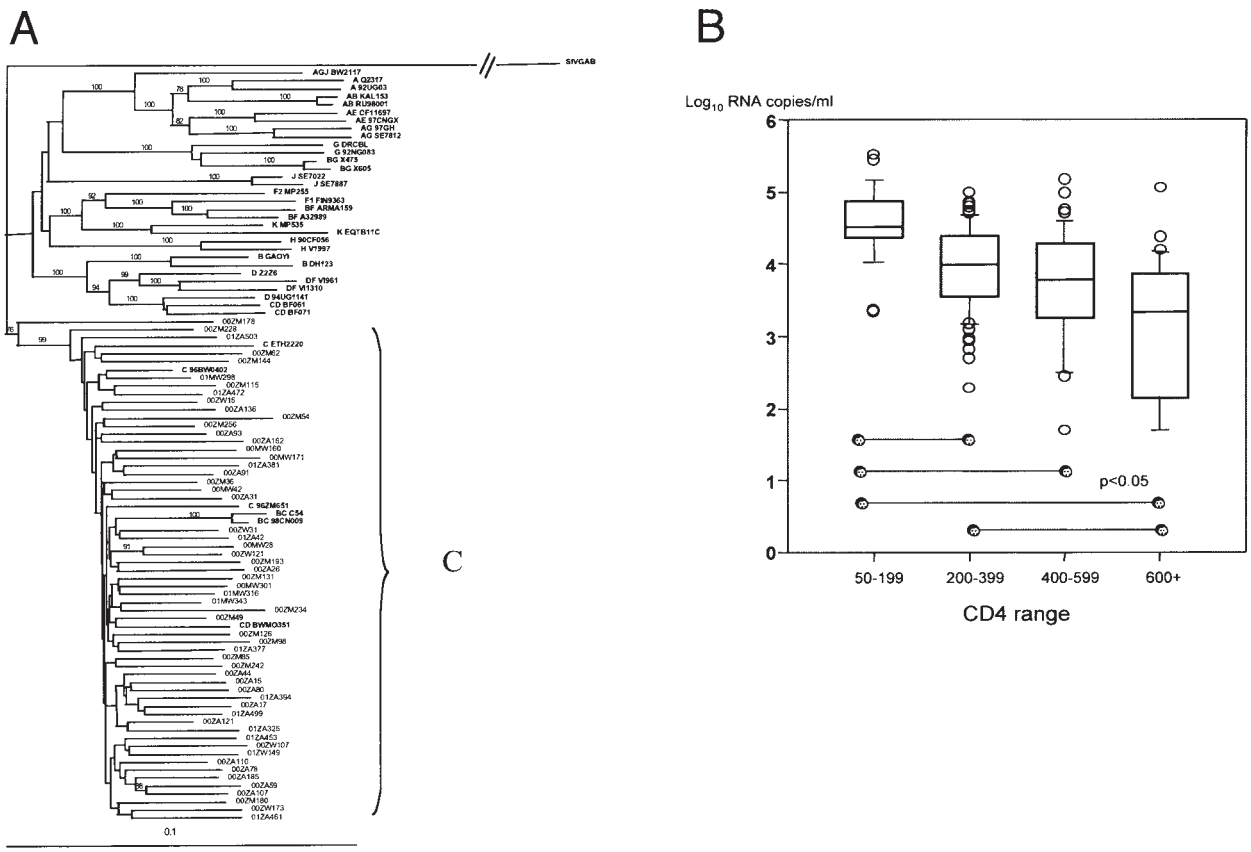

FIG. 1. (**A**) Neighbor-joining tree indicating the phylogenetic relationships between 64 full-length *gag* HIV-1 sequences (1500 bp) with 38 sequences (indicated in boldface) representing HIV-1 subtypes A–K, circulating recombinant forms (CRFs), and SIVCPZGAB. Bootstrap values >75% are shown and 63 of the sequences (Malawi, MW prefix; Zambia, ZM prefix; Zimbabwe, ZW prefix; South Africa [Durban and Johannesburg], ZA prefix) were confirmed as subtype C and 1 sequence (00ZM178) as an outlier. (**B**) Stratification of $CD4^+$ cell counts, showing statistically significant differences in viral loads between each $CD4^+$ stratum.

$p < 0.0001$), suggesting that the gross immune status of individuals, as measured by absolute $CD4^+$ cell counts, was directly related with HIV-1 viremia as observed in subtype B cohorts.[19] The data from seronegative controls in this study suggest that levels of $CD4^+$ cells in the general population in southern Africa are relatively lower than in HIV-uninfected individuals in North America or Europe. This lower trend has also been observed in Tanzania,[20] where median $CD4^+$ cell counts in HIV-1-seronegative blood donors was 800 cells/mm^3 (403–1604, IQR). It has been shown in subtype C HIV-1-infected Indians from sexually transmitted disease (STD) clinics that $CD4^+$ cell counts decline fairly early after infection, suggestive of rapid disease progression,[21] although levels of viremia appeared similar to our study. The meaning of the lower $CD4^+$ cell counts is unclear and warrants further analysis in larger cohorts to understand what impact this might have on HIV-1 infection and disease progression.

We applied a basic model of viral dynamics[22,23] to estimate the magnitude of the viral set point for each participant, taking into consideration viral load and parallel $CD4^+$ cell counts. The set point was defined as viral load changes not exceeding 0.5 $\log_{10}$ variation on at least two consecutive time points. The model consisted of three variables: the population sizes of uninfected cells (T), the mathematically implied number of infected cells (T^*), and free virus particles (v). The implementation of this model used the following assumptions; (1) that each participant of the study reached a steady viral state; (2) that participants had not reached a viremic steady state at the first measured time point; (3) that a direct interaction between virus and $CD4^+$ cells occurred; (4) that the efficiency of $CD4^+$ cell infection by subtype C HIV-1 approximated the assumed infectivity for subtype B; and (5) $CD4^+$ cell counts never exceeded the upper limits found for HIV-seronegative controls. Free virus particles infect uninfected cells at a rate proportional to their abundance, βTv, where the rate constant β describes the efficiency of infection. The assumed number of infected cells produces free virus particles at a rate proportional to their abundance, kT^*. Infected cells die at a rate μT^*, and free virus particles are removed from the system at a rate cv. By assuming a production rate s and death rate dT for the infected cells, the three-dimensional model of virus dynamics is obtained as

$$\dot{T} = s - DT - \beta Tv$$

$$\dot{T} = \beta Tv - \mu T^*$$

$$\dot{v} = kT^* - cv$$

Set point, in this context, was defined as the point with zero speed of change for all variables. Thus the viral set point was solved by $v^* = d(ks\beta/\mu dc - 1)\beta$. Forty-four of the 51 participants had minimum requirements for parameter estimation that consisted of either four or five $CD4^+$ cell and parallel viral load measurements. The Nelder–Mead search method[24,25] was used to find the best fit of the model for each data point. A curve for each participant was rooted by the first actual viral load data point and the trajectory of viral loads for each participant was made (Fig. 2). The calculated median time to set point was projected to be 16.57 (12.7–22.5, IQR) months and the median set point distribution was 4.08 $\log_{10}$ (3.59–4.42, IQR) RNA copies/ml. The fluctuations observed within the first 17 months after seroconversion were a product of viral load and $CD4^+$ cell changes for each participant. One of the limitations of this model is that it assumes that a steady state had been reached at some time after the first measurement, which may not be strictly the case with some participants in the study. Nonetheless, these data show that there is greater variability in viremia during the early phase of infection. Although the primary goal of an HIV-preventive vaccine is to induce sterilizing immunity, this may not be attained, and an important secondary marker of vaccine effectiveness would be the impact of vaccines on viral set point. From this perspective, our data would suggest that it might be prudent to assess vaccine effects on viral load beyond 17 months, when reduced fluctuations in viremia would be more apparent.

A number of published studies reporting viral loads have used different assays to measure viral loads. To compare our viral load estimations with other cohorts we replicated measurements using different assay platforms. Selected samples were sent blinded to two external laboratories for measurement of RNA copies either by the NucliSens (NASBA) assay (lower limit of 125 copies/ml) or the ultrasensitive Roche Amplicor 1.5 (lower limit of 20 copies/ml). Additional matched samples stored on site in Zimbabwe were also measured by Roche Amplicor 1.5 (lower limit of 400 copies/ml) to discount possible degradation of virus due to shipment. Figure 3A shows a significant correlation ($r = 0.81$; $p < 0.0001$) between bDNA and NucliSens (NASBA) measurements, although six samples with fewer than 125 copies/ml, using NASBA, corresponded to a geometric mean of 2143 RNA copies/ml (range, 1241–3876 copies/ml), using bDNA. When samples were compared with the standard Roche Amplicor 1.5 assay (lower detection of 400 copies/ml), there was a highly significant correlation (Fig. 3B; $r = 0.87$; $p < 0.0001$), although the standard Roche Amplicor 1.5 was consistently measuring a median of 0.57 $\log_{10}$ RNA copies/ml greater than bDNA. There was also a significant correlation between bDNA with the ultrasensitive Roche Amplicor assay ($n = 20$) of $r = 0.91$ ($p < 0.0001$), although ultrasensitive RT-PCR was consistently measuring a median of 0.32 $\log_{10}$ RNA copies/ml greater than bDNA. When we correlated viral load values between those performed on site in Zimbabwe with

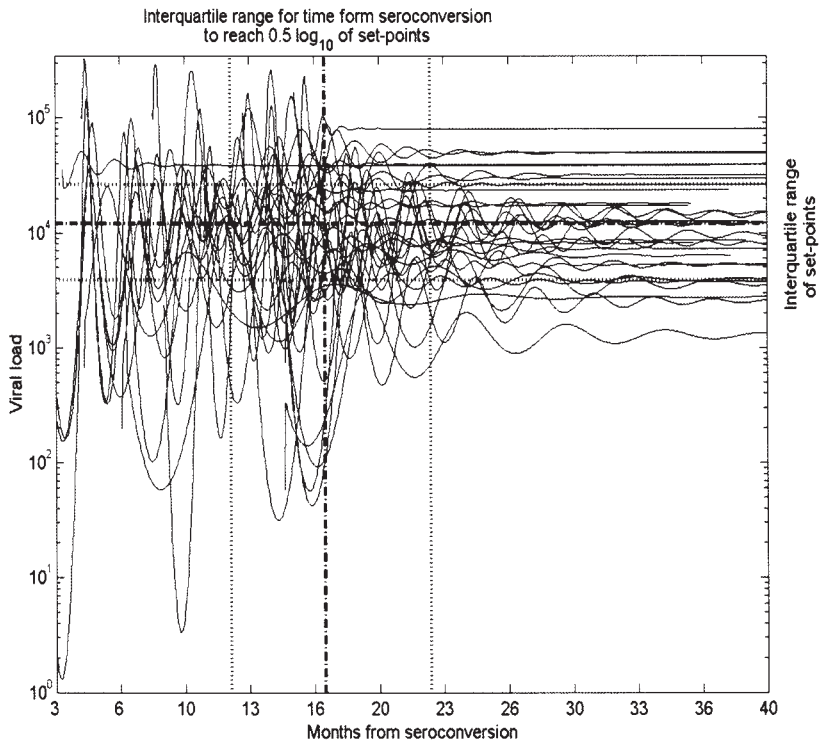

FIG. 2. Curve fitting of $\log_{10}$ viral load trajectories, using a basic mathematical model for 44 of 51 participants who had the minimum requirements for parameter estimation, where the viral set point was solved by $v^* = d(ks\beta/\mu dc - 1)/\beta$. The plot shows the interquartile ranges for both the time to set point and the final set point. Curve fitting was done as described[25] and the best fit was defined in terms of square distance between actual data and modeled data, while taking into account two further assumptions: (1) $CD4^+$ cell count upper limit never exceeded 1200 cells/mm^3 and (2) death rate of infected cells (μ) was less than the death rate of virus (c).

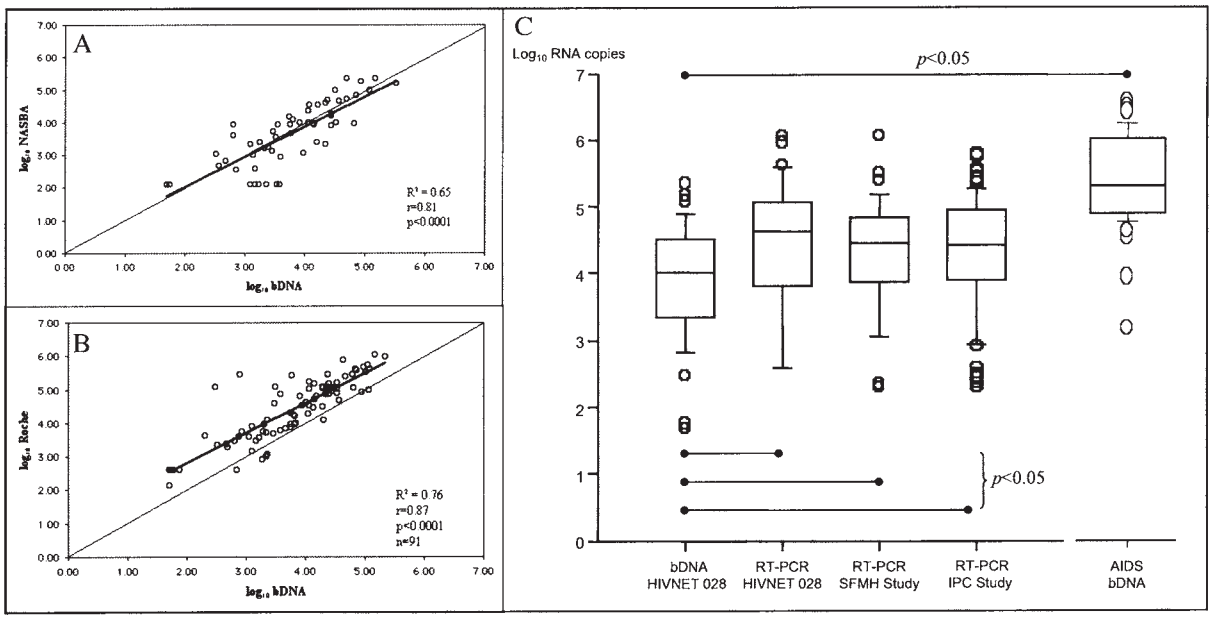

FIG. 3. (**A**) Correlation between $\log_{10}$ bDNA and $\log_{10}$ NASBA viral load measurements on blinded replicate samples (n = 61). (**B**) Correlation between $\log_{10}$ bDNA and $\log_{10}$ RT-PCR (Roche Amplicor) viral load measurements on blinded replicate samples (n = 91). (**C**) Comparison of viral load measurements made using bDNA and RT-PCR (Roche) on replicate samples with viral load measurements made in subtype B HIV-1-infected cohorts from the San Francisco Men's Health (SFMH) Study and Infected Participants Cohort (IPC) and compared with subtype C HIV-1-infected patients with AIDS (n = 44).

the shipped counterparts in South Africa (n = 30), there was a significant correlation of r = 0.85 ($p < 0.0001$). In this case, the Roche Amplicor and bDNA assays were comparing freshly stored plasma with matched shipped samples, with RT-PCR measuring a median of 0.59 $\log_{10}$ greater than bDNA. Collectively, these data demonstrate that RT-PCR was detecting significantly ($p < 0.001$) higher subtype C viremia than bDNA, in agreement with other studies.[26,27]

These comparative data allowed us to compare the magnitude of viremia in the HIVNET 028 cohort with subtype B-infected adult cohorts that used the Roche assay. Figure 3C shows comparisons between replicate subtype C viral load measurements using bDNA and Roche in the HIVNET 028 study within the first 12 months of infection (n = 50) with subtype B viral load measurements from the San Francisco Men's Health Study (SFMHS)[28] and Infected Participant Cohort.[3] These data show that subtype C viral loads measured with the Roche Amplicor assay were comparable with recently infected subtype B HIV-1-infected male individuals, when they were matched for the time after seroconversion. The geometric mean of subtype C viral loads within the first 12 months from midpoint were 27,791 RNA copies/ml; SFMHS was 17,542 RNA copies/ml (n = 35); and the IPC was 18,165 copies/ml (n = 104). There were no significant differences. By comparison, the bDNA assay measured a geometric mean of 7366 RNA copies/ml for the same period. To confirm whether the bDNA assay was able to measure high viral loads in subtype C HIV infection, a cohort of AIDS patients (n = 44) was analyzed, showing significantly higher geometric mean viremia of 254,451 copies/ml.

It has been shown that HIV subtype may impact on the course of disease, where (e.g., in Uganda) subtype D was associated with faster disease progression compared with subtype A.[29] In Senegal, subtype A HIV-1-infected individuals were more likely to have slower disease progression than those infected with non-A subtypes, who progressed more rapidly to AIDS.[30] In India, there was no distinction between subtype C viral loads and that found in subtype B infection, although differences in viral load trajectory suggested a more rapid disease course.[21] Conversely, other studies found no association between subtype and disease progression.[31,32] In our study, the estimated viral load set point for subtype C in southern Africa suggests that subtype C HIV-1 infection in Africa may follow the same disease course as subtype B infection as described in cohorts from North America and Europe. The lack of longer term follow-up in our cohort precludes us from making conclusions about viral trajectory and disease progression beyond the first 24 months after seroconversion. There are several caveats in making our conclusions. First, the composition of the cohort was predominantly female. It has been shown that HIV-1 viremia in women at the early stages of infection is significantly lower than in males,[33,34] although disease progression is no different between genders.[35] The possibility that viral loads were low because of assay effects was excluded by replicating measurements with different assays. Furthermore, the incidence of coinfections, such as with *Mycobacterium tuberculosis*, may significantly alter the course of HIV-1 disease[36] independently of $CD4^+$ cell counts and viral load measurements in early infection.

In conclusion, the dynamics and attainment of set point in subtype C HIV infection in southern Africa appears no different from that found for subtype B infection and the course of plasma viremia, as described in this study, will allow a useful baseline comparator for the effects of HIV-1 vaccines.

ACKNOWLEDGMENTS

Financial support was received from NIH grant N01-AI-45202 (HIVNET 028) and the South African AIDS Vaccine Initiative (SAAVI).

The authors acknowledge the contributions of the investigators, site coordinators, clinicians, and other staff at each HIV Network for Prevention Trials (HIVNET) site, and the participation of the study participants. They especially acknowledge the effort of the study coordinators and monitors. Acknowledgments are also extended to the HIV Vaccine Trials Network Publications Committee for useful feedback.

Members of the HIVNET 028 team: Clive Gray (co-chair), Haynes Sheppard (co-chair); *Zambia*—Rosamary Masondu, Susan Allen, Michelle Klautzman; *Malawi*—Taha Taha, Newton Kumwenda; *Zimbabwe*—David Katzenstein, Lynn Zijenah, Victoria Aquino, Michael Chirenje, Mike Mbizo, Ocean Tobaiwa; *South Africa*: Johannesburg—Glenda Gray, James McIntyre, Armstrong Mafhandu; Durban—Efthyia Vardas, Mark Colvin, Dudu Msweli, Wendy Dlamini, Gita Ramjee, Salim Abdool Karim, Quarraisha Abdool Karim; NICD—Lynn Morris, Natasha Taylor; University of Cape Town—Carolyn Williamson, Helba Bredell, Celia Rademayer; Jorge Flores (DAIDS); Ward Cates, Linda McNeil, Missie Allen (Family Health International).

The authors acknowledge the expert technical help provided in the laboratory by Greg Khoury, Phineas Mohube, Pauline Mokgotho, Moses Mashiloane, Japh Sibeko, Precious Magooa, and Ewalde Cutler.

REFERENCES

1. Henrad D, Phillips J, Muenz L, *et al.*: Natural history of HIV-1 cell-free viremia. JAMA 1995;274:554–558.
2. Hu DJ, Subbarao S, Vanichseni S, *et al.*: Higher viral loads and other risk factors associated with HIV-1 seroconversion during a period of high incidence among injection drug users in Bangkok. J Acquir Immune Defic Syndr 2002;30:240–247.
3. Celum CL, Buchbinder SP, Donnell D, *et al.*: Early human immunodeficiency virus (HIV) infection in the HIV Network for Prevention Trials Vaccine Preparedness Cohort: Risk behaviors, symptoms, and early plasma and genital tract virus load. J Infect Dis 2001;183:23–35.
4. Muller V, Maree AF, and De Boer RJ: Small variations in multiple parameters account for wide variations in HIV-1 set-points: A novel modelling approach. Proc R Soc Lond B Biol Sci 2001;268:235–242.
5. de Wolf F, Spijkerman I, Schellekens PT, *et al.*: AIDS prognosis based on HIV-1 RNA, $CD4^+$ T-cell count and function: Markers with reciprocal predictive value over time after seroconversion. AIDS 1997;11:1799–806.
6. Lyles RH, Munoz A, Yamashita TE, *et al.*: Natural history of human immunodeficiency virus type 1 viremia after seroconversion and proximal to AIDS in a large cohort of homosexual men. Multicenter AIDS Cohort Study. J Infect Dis 2000;181:872–880.
7. Masel J, Arnaout RA, O'Brien TR, Goedert JJ, and Lloyd AL: Fluctuations in HIV-1 viral load are correlated to $CD4^+$ T-lymphocyte count during the natural course of infection. J Acquir Immune Defic Syndr 2000;23:375–379.
8. Sabin CA, Devereux H, Phillips AN, *et al.*: Course of viral load throughout HIV-1 infection. J Acquir Immune Defic Syndr 2000;23:172–177.
9. Vidal C, Garcia F, Romeu J, *et al.*: Lack of evidence of a stable viral load set-point in early stage asymptomatic patients with chronic HIV-1 infection. AIDS 1998;12:1285–1289.
10. Schacker TW, Hughes JP, Shea T, Coombs RW, and Corey L: Biological and virologic characteristics of primary HIV infection. Ann Intern Med 1998;128:613–620.
11. Salamon R, Marimoutou C, Ekra D, *et al.*: Clinical and biological evolution of HIV-1 seroconverters in Abidjan, Côte d'Ivoire, 1997–2000. J Acquir Immune Defic Syndr 2002;29:149–157.
12. Andersson S, Norrgren H, da Silva Z, *et al.*: Plasma viral load in HIV-1 and HIV-2 singly and dually infected individuals in Guinea-Bissau, West Africa: Significantly lower plasma virus set point in HIV-2 infection than in HIV-1 infection. Arch Intern Med 2000;160:3286–3293.
13. Richardson BA, Mbori-Ngacha D, Lavreys L, *et al.*: Comparison of human immunodeficiency virus type 1 viral loads in Kenyan women, men, and infants during primary and early infection. J Virol 2003;77:7120–7123.
14. Nkengasong JN, Kestens L, Ghys PD, *et al.*: Human immunodeficiency virus Type 1 (HIV-1) plasma virus load and markers of immune activation among HIV-infected female sex workers with sexually transmitted diseases in Abidjan, Côte d'Ivoire. J Infect Dis 2001;183:1405–1408.
15. Grobler J, Gray C, Seoighe C, *et al.*: The incidence of HIV-1 dual infection and its association with increased viral load set point in a chort of subtype C infected female sex-workers. J Infect Dis 2004;190:1355–1359.
16. Rinke de Wit TF, Tsegaye A, Wolday D, *et al.*: Primary HIV-1 subtype C infection in Ethiopia. J Acquir Immune Defic Syndr 2002;30:463–470.
17. Taha TE, Kumwenda NI, Hoover DR, *et al.*: Association of HIV-1 load and CD4 lymnphocyte count with mortality among untreated African children over one year of age. AIDS 2000;14:453–459.
18. Williamson AL: The development of HIV-1 subtype C vaccines for southern Africa. IUBMB Life 2002;53:207–208.
19. Mellors JW, Rinaldo CR Jr, Gupta P, White RM, Todd JA, and Kingsley LA: Prognosis in HIV-1 infection predicted by the quantity of virus in plasma. Science 1996;272:1167–1170.
20. Urassa WK, Mbena EM, Swai AB, Gaines H, Mhalu FS, and Biberfeld G: Lymphocyte subset enumeration in HIV seronegative and HIV-1 seropositive adults in Dar es Salaam, Tanzania: Determination of reference values in males and females and comparison of two flow cytometric methods. J Immunol Methods 2003;277:65–74.
21. Mehendale SM, Bollinger RC, Kulkarni SS, *et al.*: Rapid disease progression in human immunodeficiency virus type 1-infected seroconverters in India. AIDS Res Hum Retroviruses 2002;18:1175–1179.
22. Herz AV, Bonhoeffer S, Anderson RM, May RM, and Nowak MA: Viral dynamics *in vivo*: Limitations on estimates of intracellular delay and virus decay. Proc Natl Acad Sci USA 1996;93:7247–7251.
23. Nowak MA and Bangham CR: Population dynamics of immune responses to persistent viruses. Science 1996;272:74–79.
24. Lagarias J, Reeds J, Wright M, and Wright P: Convergence properties of the Nelder–Mead simplex method in low dimensions. J Optimization 1998;9:112–147.
25. Filter R, Xia X, and Gray CM: Dynamic HIV/AIDS parameter estimation with application to a vaccine readiness study in South Africa. IEEE Trans Automatic Control 2004 (in press).
26. Amendola A, Bordi L, Angeletti C, *et al.*: Underevaluation of HIV-1 plasma viral load by a commercially available assay in a cluster of patients infected with HIV-1 A/G circulating recombinant form (CRF02). J Acquir Immune Defic Syndr 2002;31:488–494.
27. Burgisser P, Vernazza P, Flepp M, *et al.*: Performance of five different assays for the quantification of viral load in persons infected

with various subtypes of HIV-1. Swiss HIV Cohort Study. J Acquir Immune Defic Syndr 2000;23:138–144.
28. Lee TH, Sheppard HW, Reis M, Dondero D, Osmond D, and Busch MP: Circulating HIV-1-infected cell burden from seroconversion to AIDS: Importance of postseroconversion viral load on disease course. J Acquir Immune Defic Syndr 1994;7:381–388.
29. Kaleebu P, French N, Mahe C, *et al*.: Effect of human immunodeficiency virus (HIV) type 1 envelope subtypes A and D on disease progression in a large cohort of HIV-1-positive persons in Uganda. J Infect Dis 2002;185:1244–1250.
30. Kanki PJ, Hamel DJ, Sankale JL, *et al*.: Human immunodeficiency virus type 1 subtypes differ in disease progression. J Infect Dis 1999;179:68–73.
31. Alaeus A, Lidman K, Bjorkman A, Giesecke J, and Albert J: Similar rate of disease progression among individuals infected with HIV-1 genetic subtypes A–D. AIDS 1999;13:901–907.
32. Laurent C, Bourgeois A, Faye MA, *et al*.: No difference in clinical progression between patients infected with the predominant human immunodeficiency virus type 1 circulating recombinant form (CRF) 02_AG strain and patients not infected with CRF02_AG, in western and West-Central Africa: A four-year prospective multicenter study. J Infect Dis 2002;186:486–492.
33. Napravnik S, Poole C, Thomas JC, and Eron JJ Jr: Gender difference in HIV RNA levels: A meta-analysis of published studies. J Acquir Immune Defic Syndr 2002;31:11–19.
34. Sterling TR, Lyles CM, Vlahov D, Astemborski J, Margolick JB, and Quinn TC: Sex differences in longitudinal human immunodeficiency virus type 1 RNA levels among seroconverters. J Infect Dis 1999;180:666–672.
35. Sterling TR, Vlahov D, Astemborski J, Hoover DR, Margolick JB, and Quinn TC: Initial plasma HIV-1 RNA levels and progression to AIDS in women and men. N Engl J Med 2001;344:720–725.
36. Lawn SD: AIDS in Africa: The impact of coinfections on the pathogenesis of HIV-1 infection. J Infect 2004;48:1–12.

Address reprint requests to:
Clive Gray
HIV Immunology Laboratory AIDS Unit
National Institute for Communicable Diseases
Johannesburg, South Africa 2131

E-mail: cgray@nicd.ac.za