

Quantitative Analysis of Syncytium-Inducing and Non-Syncytium-Inducing Virus in Patients Infected with Human Immunodeficiency Virus Type 1

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Among 75 consecutive human immunodeficiency virus type 1 (HIV-1)-infected patients with moderate and advanced immunosuppression, those harboring syncytium-inducing (SI) HIV-1 had a lower CD4⁺-cell count (145 versus 278 cells per μ l, $P < 0.001$) and 10-fold-higher virus titers than patients with non-SI HIV-1 (398 versus 39 infectious units per 10^6 CD4⁺ lymphocytes; $P < 0.001$). In patients with SI virus, the mean titer of SI virus, determined with a quantitative MT-2 cell assay, was 135 SI infectious units per 10^6 CD4⁺ lymphocytes. Virus titer correlated inversely with CD4⁺-cell count in patients with SI ($r = -0.67$) but not non-SI ($r = -0.29$) virus.

Virus burden and phenotype both correlate with severity of immunosuppression in patients infected with human immunodeficiency virus type 1 (HIV-1). Patients with advanced disease are likely to have a higher virus burden (2-4, 6) and to harbor HIV-1 strains that induce syncytia in CD4⁺ lymphoblastoid cell lines (syncytium inducing [SI]) (1, 12). High virus burden and SI phenotype are also each predictive of disease progression (2, 4, 7).

To study the relationship of virus burden to the SI phenotype, we quantified the total number of infectious units and the number of SI units per million patient CD4⁺ T lymphocytes. The titer of infectious virus was measured by culturing serial dilutions of patient peripheral blood mononuclear cells (PBMC) in phytohemagglutinin-stimulated PBMC from HIV-seronegative individuals (donor PBMC) (5). The titer of SI virus was measured by culturing serial dilutions of patient PBMC in MT-2 cells, a CD4⁺ lymphoblastoid cell line. Donor PBMC support the growth of SI and non-SI (NSI) strains, whereas MT-2 cells only support the growth of SI strains (8).

Methods. After patients gave informed consent, blood samples were obtained from 75 consecutive HIV-1-seropositive patients with moderate and advanced immunodeficiency participating in clinical trials. PBMC were isolated by Ficoll-Hypaque centrifugation, washed, and counted. Six serial five-fold dilutions of patient PBMC (10^6 , 2×10^5 , 4×10^4 , 8×10^3 , 1.6×10^3 , and 3.2×10^2 cells) were cultured in an MT-2 cell assay to quantify SI strains and in a donor PBMC assay to quantify all infectious HIV-1 strains (SI and NSI).

In the donor PBMC assay, the dilutions of patient PBMC were cocultured in duplicate with 10^6 phytohemagglutinin-stimulated donor PBMC. Cultures were propagated in RPMI 1640 medium with L-glutamine supplemented with 15% heat-inactivated fetal bovine serum (Intergen, Purchase, N.Y.), interleukin 2 (25 IU/ml; Cetus, Emeryville, Calif.), penicillin (100 U/ml), and streptomycin (100 μ g/ml). A 50% medium exchange was performed twice weekly, and 500,000 donor PBMC were added to each culture well after 7 days. After 14 days, the HIV-1 p24 antigen concentration was measured in

each culture well by enzyme immunoassay (Abbott, Chicago, Ill.), and wells with ≥ 100 pg/ml were considered positive.

In the MT-2 cell assay, the dilutions of patient PBMC were cocultured in duplicate with approximately 1×10^5 to 2×10^5 MT-2 cells. Cultures were propagated in the medium described above without interleukin 2. An 80% medium exchange was performed twice weekly. Wells were examined twice weekly, and those with syncytia by day 14 were considered positive.

The titer of infectious virus, or 50% tissue culture infective dose, per 10^6 patient PBMC was calculated for each assay with the Karber formula (9). The 50% tissue culture infective dose per 10^6 CD4⁺ lymphocytes was calculated by correcting the 50% tissue culture infective dose per 10^6 PBMC for the percent patient CD4⁺ lymphocytes. The Student *t* test was used to compare the absolute value of CD4⁺-cell counts and the log₁₀ titers of infectious virus between groups of patients. The Pearson correlation coefficients were also calculated with the log₁₀ titers of infectious virus.

Control experiments with purified stocks of SI virus (strains IIB and AO8-H112, obtained from the AIDS Reference and Reagent Registry, National Institute of Allergy and Infectious Diseases) titrated in MT-2 cells and donor PBMC demonstrated no difference in the capacity of SI strains to grow in MT-2 cells compared with that in donor PBMC (data not shown).

Results. High virus burden and SI phenotype were both associated with advanced immunosuppression. Virus burden, determined with the donor PBMC assay, correlated inversely with CD4⁺-cell count ($r = -0.47$), and of 75 patients, the 25 (33%) with an SI phenotype had a lower mean CD4⁺-cell count than the 50 (67%) with an NSI phenotype (145 versus 278 cells per μ l; $P < 0.001$).

To compare virus burdens of patients with SI and NSI phenotypes, we examined the titer of infectious virus in the donor PBMC assay for both groups of patients. The 25 patients harboring virus with the SI phenotype had a 10-fold-higher total virus burden than the 50 patients harboring virus with the NSI phenotype (398 versus 39 infectious units per 10^6 patient CD4⁺ lymphocytes [$P < 0.001$]; 95% confidence interval, 3- to 27-fold increase in virus burden [SI virus group versus NSI virus group]) (Fig. 1). In a multiple linear regression analysis, low CD4⁺-cell count ($p = 0.001$) and SI phenotype ($P = 0.03$) were both independently associated with higher total virus

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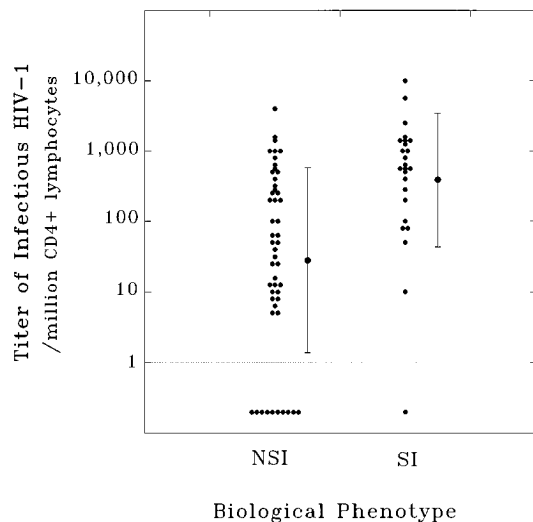


FIG. 1. Titers of infectious HIV-1 per 10^6 CD4⁺ lymphocytes in 25 patients with SI virus and in 50 patients with NSI virus. Titers were determined in the donor PBMC assay, which is permissive for both SI and NSI strains. Patients with SI virus had a 10-fold-higher virus burden than patients with NSI virus (398 versus 39 infectious units per 10^6 patient CD4⁺ lymphocytes [$P < 0.001$]; 95% confidence interval, 3- to 27-fold increase in virus burden).

burden. Thus, SI phenotype and high virus burden correlated with each other as well as with CD4⁺-cell depletion.

We examined the effect of virus phenotype on the correlation of virus titer with CD4⁺-cell count. Among the 25 patients with SI virus, there was a strong inverse correlation between virus titer and CD4⁺-cell count ($r = -0.67$) (Fig. 2A). In contrast, among the 50 patients with NSI virus, there was a weak inverse correlation between virus titer and CD4⁺-cell count ($r = -0.26$) (Fig. 2B).

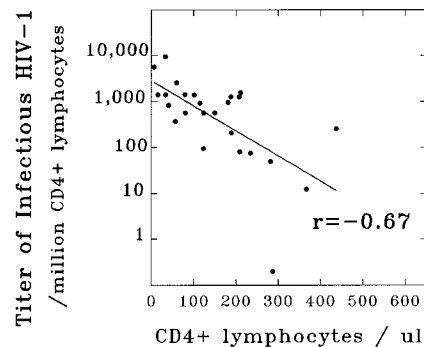
To ascertain the relative proportions of SI and NSI viruses in patients with the SI phenotype, we compared the titers of SI virus obtained with the MT-2 cell assay with the titers of all infectious virus obtained with the donor PBMC assay. The mean titer of SI virus (obtained with the MT-2 cell assay) in the 25 patients with the SI phenotype was 135 infectious units per 10^6 CD4⁺ T lymphocytes (range, 5 to 3,125). In contrast, the mean virus titer (obtained with the donor PBMC assay) in these 25 patients was 398 infectious units per 10^6 CD4⁺ lymphocytes.

Discussion. In this group of HIV-1-infected patients with moderate and advanced immunosuppression, we have confirmed that SI phenotype and high virus burden are each associated with CD4⁺-lymphocyte depletion. In addition, we have observed a 10-fold-higher virus burden in the 25 patients with SI virus than in the 50 patients without SI virus. Virus burden and SI phenotype, therefore, are codependent variables, each also correlating with the severity of immunosuppression.

The titer of infectious virus was calculated per 10^6 patient CD4⁺ lymphocytes, because nearly all circulating proviral HIV-1 is found within CD4⁺ lymphocytes (10, 13). The virus titers determined by the donor PBMC and MT-2 cell assays at 14 days may underestimate the circulating virus burden. Culture techniques appear less sensitive in detecting HIV-1 than quantitative DNA PCR techniques (10, 13). Moreover, higher virus titers might have been obtained if quantitative cultures had been incubated for even longer periods.

Quantitative assays of HIV-1 are increasingly being used to

A



B

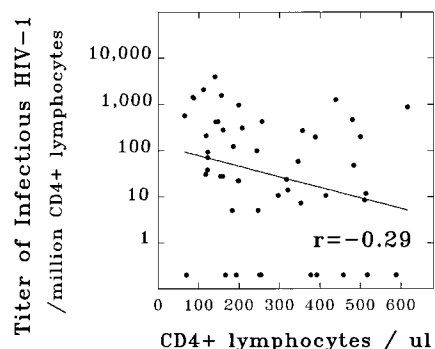


FIG. 2. Correlation of HIV-1 titer with CD4⁺-lymphocyte count among 25 patients with SI virus (A) and 50 patients with NSI virus (B). The titer of infectious virus, or 50% tissue culture infective dose, per 10^6 patient PBMC was calculated for each assay with the Karber formula (9).

monitor virus burden in HIV-1-infected patients (5). Our finding, that virus titer correlated inversely with CD4⁺-cell count in patients with SI but not NSI virus, requires confirmation with a larger number of patients. A poor correlation between virus titer and CD4⁺-cell count in patients with NSI virus may reflect the presence of variables in addition to circulating virus burden which are influencing the rate of CD4⁺-lymphocyte depletion.

Previously described methods for detecting the SI phenotype have been qualitative. Therefore, it has not been known whether SI strains constitute a small fraction or the majority of viruses in patients with the SI phenotype. Indeed, Schuitemaker et al. have tested biological clones from a patient harboring virus with the SI phenotype and shown that NSI strains were also present (11). By using a quantitative assay for SI virus, we have shown that in patients with the SI phenotype, many SI infectious units may be present. However, the titer of SI virus in MT-2 cells was generally lower than the titer of infectious virus in donor PBMC. One explanation for this result is the persistence of a large proportion of NSI strains in patients with the SI phenotype. Alternatively, MT-2 cells may be less susceptible than donor PBMC to infection, even with SI virus strains. In both cases, SI strains constitute more than a small fraction of infectious viruses in patients with the SI phenotype.

REFERENCES

1. Bozette, S. A., J. A. McCutchan, S. A. Spector, B. Wright, and D. D. Richman. 1993. A cross-sectional comparison of persons with syncytium- and

- non-syncytium-inducing human immunodeficiency virus. *J. Infect. Dis.* **168**:1374–1379.
2. **Connor, R. I., H. Mohri, Y. Cao, and D. D. Ho.** 1993. Increased viral burden and cytopathicity correlate temporally with CD4⁺ T-lymphocyte decline and clinical progression in human immunodeficiency type 1-infected individuals. *J. Virol.* **67**:1772–1777.
 3. **Coombs, R. W., A. C. Collier, J. P. Allain, B. Nikora, M. Leuther, G. F. Gjerset, and L. Corey.** 1989. Plasma viremia in patients with human immunodeficiency virus. *N. Engl. J. Med.* **321**:1626–1631.
 4. **Gupta, P., L. Kingsley, J. Armstrong, M. Ding, M. Cottrill, and C. Rinaldo.** 1993. Enhanced expression of human immunodeficiency virus type 1 correlates with development of AIDS. *Virology* **196**:586–595.
 5. **Hammer, S., C. Crumpacker, R. D'Aquila, B. Jackson, J. Lathey, D. Livnat, and P. Reichelderfer.** 1993. Use of virologic assays for detection of human immunodeficiency virus in clinical trials: recommendations of the AIDS Clinical Trials Group Virology Committee. *J. Clin. Microbiol.* **31**:2557–2564.
 6. **Ho, D. D., T. Moudgil, and M. Alam.** 1989. Quantitation of human immunodeficiency virus type 1 in blood of infected persons. *N. Engl. J. Med.* **321**:1621–1625.
 7. **Koot, M., I. P. M. Keet, A. H. V. Vos, R. E. Y. de Goede, M. T. L. Roos, R. A. Coutinho, F. Miedema, P. T. A. Schellekens, and M. Tersmette.** 1993. Prognostic value of HIV-1 syncytium-inducing phenotype for rate of CD4⁺ cell depletion and progression to AIDS. *Ann. Intern. Med.* **118**:681–688.
 8. **Koot, M., A. H. V. Vos, I. P. M. Keet, R. E. Y. de Goede, M. W. Dercksen, F. G. Terpstra, R. A. Coutinho, F. Miedema, and M. Tersmette.** 1992. HIV-1 biological phenotype in long-term infected individuals evaluated with an MT-2 co-cultivation assay. *AIDS* **6**:49–54.
 9. **Schmidt, N. J.** 1989. Cell culture procedures for diagnostic virology, p. 78–79. In N. J. Schmidt and R. W. Emmons (ed.), *Diagnostic procedures for viral, rickettsial, and chlamydial infections*. American Public Health Association, Washington, D.C.
 10. **Schnittman, S. M., M. C. Psallidopoulos, H. C. Lane, L. Thompson, M. Baseler, F. Massari, C. H. Fox, N. P. Salzman, and A. S. Fauci.** 1989. The reservoir of HIV-1 in peripheral blood is a T cell that maintains expression of CD4. *Science* **245**:305–308.
 11. **Schuitemaker, H., M. Koot, N. A. Kootstra, M. W. Dercksen, R. E. Y. de Goede, R. P. van Steenwijk, J. M. A. Lange, J. K. M. E. Schattenkerk, F. Miedema, and M. Tersmette.** 1992. Biological phenotype of human immunodeficiency virus type 1 clones at different stages of infection: progression of disease is associated with a shift from monocytopathic to T-cell-tropic virus populations. *J. Virol.* **66**:1354–1360.
 12. **Tersmette, M., R. A. Gruters, F. de Wolf, R. E. Y. de Goede, J. M. A. Lange, P. T. A. Schellekens, J. Goudsmit, H. G. Huisman, and F. Miedema.** 1989. Evidence for a role of virulent human immunodeficiency virus (HIV) variants in the pathogenesis of acquired immunodeficiency syndrome: studies on sequential HIV isolates. *J. Virol.* **63**:2118–2125.
 13. **Wood, R., H. Dong, D. A. Katzenstein, and T. C. Merigan.** 1993. Quantification and comparison of HIV-1 proviral load in peripheral blood mononuclear cells and isolated CD4⁺ T cells. *J. Acquired Immune Defic. Syndr.* **6**:237–240.