Restoration of Cell Surface CD4 Expression in Human Immunodeficiency Virus Type 1-Infected Cells by Treatment with a Tat Antagonist

MUHAMMAD SHAHABUDDIN,¹ BARBARA VOLSKY,¹ MING-CHU HSU,² AND DAVID J. VOLSKY^{1*}

Molecular Virology Laboratory, St. Luke's/Roosevelt Hospital Center and The College of Physicians and Surgeons, Columbia University, Antenucci Building, 432 West 58th Street, New York, New York 10019,¹ and Department of Virology, Hoffmann La Roche, Nutley, New Jersey 07110²

Received 22 May 1992/Accepted 3 August 1992

Productive infection of T lymphocytes with human immunodeficiency virus type 1 (HIV-1) is accompanied by a diminution of surface CD4 receptors. Treatment of chronically HIV-1-infected CD4-negative T cells in vitro with the Tat antagonist Ro 5-3335 resulted in a drug dose-dependent decrease in virus protein production and a reciprocal increase in surface CD4 display. The drug-treated cells remained viable, showed significantly reduced levels of the full-length and spliced HIV-1 mRNAs as detected by Northern (RNA) blot hybridization, and maintained integrated HIV-1 DNA. In immunoprecipitation studies with drug-treated cells, the levels of free 55-kDa CD4 protein increased and gp160 complexed with CD4 decreased in amount. These results show for the first time that certain cytopathogenic effects of chronic HIV-1 infection can be reversed by suppressing virus expression.

CD4 is a member of the immunoglobulin superfamily which is required for the immunologic function of helper/inducer T lymphocytes (6, 20) and also serves as the receptor for human immunodeficiency virus type 1 (HIV-1) (9, 18, 19). HIV-1 infection of T cells or monocytes in vitro usually results in a loss of surface CD4 (11-13, 21). In effect, HIV-1-infected helper/inducer T cells incur damage at two levels: they lose immunologic signal molecules (that is, CD4), and they may perish as a result of viral replication. Both these effects can contribute to the development of immunodeficiency and AIDS (24). The mechanisms responsible for CD4 downmodulation are not fully understood, but initial infection kinetics studies suggested a correlation between the CD4 decline and the increased expression of HIV-1 RNA and proteins (12, 27). HIV-1 infection has little effect on the steady-state levels of CD4 mRNA (11-13, 16, 17, 27), indicating that CD4 downmodulation by HIV-1 is posttranscriptional. This notion is substantiated by reports showing that cells productively infected with HIV-1 accumulate intracellular complexes of viral gp160 glycoproteins and newly synthesized CD4 (11-13, 16, 17, 27), formation of which could prevent CD4 transport to the plasma membrane (1, 8, 15). Induction of latent HIV-1 in vitro causes rapid downmodulation of surface CD4, consistent with increased synthesis of viral gp160 polypeptide and the appearance of intracellular CD4-gp160 complexes (3). Taken together, these results suggest an inverse correlation between surface CD4 levels and HIV-1 expression. In this work, we tested whether the loss of surface CD4 in HIV-1-infected T cells in vitro can be reversed by inhibiting the expression of the extant HIV-1 genome. We report that treatment of chronically infected, CD4-negative T cells with the recently described HIV-1 Tat antagonist Ro 5-3335 (14) diminished the production of HIV-1 RNA and proteins and consequently increased the intracellular levels of free CD4 and partially restored surface CD4 display.

Reversible extinction of HIV-1 expression by an HIV-1 Tat antagonist in chronically infected T cells. Results of a typical Ro 5-3335 dose-response experiment using CR10/N1T cells, derivatives of CR10 cells (4) chronically and productively infected with HIV-1 strain N1T (25), are summarized in Table 1. As previously demonstrated, these cells do not display surface CD4 receptors (4) and exhibit lower levels of CD4 protein immunoprecipitable by OKT4A antibody than uninfected CR10 cells (27). CR10/N1T cells were washed, supplemented with various doses of Ro 5-3335, and cultured for 6 days under standard conditions. Cell viability was determined by trypan blue exclusion, and HIV-1 antigens were detected either by indirect immunofluorescence (IF) staining of cell smears fixed in acetone (4) or by measuring the levels of HIV-1 p24 capsid proteins in cell lysates (10×10^6 cells per ml) with the Coulter HIV-1 Ag Assay (Coulter Immunology, Hialeah, Fla.), as described previously (29). Surface CD4 was analyzed by flow cytometry in an Epics C instrument using monoclonal T4 antibody conjugated with rhodamine B isothiocyanate (Coulter Immunology). The T4 antibody does not compete for the gp120 binding site on CD4.

In agreement with our recent study (14), incubation of CR10/N1T cells with Ro 5-3335 for 6 days resulted in a drug dose-dependent decline in the production of HIV-1 p24 core proteins from over 2,000 ng/ 10^6 cells in the absence of drug to about 30 ng/ 10^6 cells in the presence of 5 μ M Ro 5-3335 (Table 1). Significant reduction in the expression of intracellular viral antigens detected by IF staining, from 66 to 2% IF-positive cells, respectively, was also observed (Table 1). The median dose-response concentration (50% inhibitory concentration) for Ro 5-3335, extrapolated from p24 data in Table 1, is about 0.5 μ M, which is within the 0.1 to 1 μ M range reported for a variety of target cells infected with various HIV-1 strains (14). Dideoxycytidine, a reverse transcription inhibitor, had no effect on HIV-1 replication in CR10/N1T cells (data not shown). Removal of Ro 5-3335 after 6 days of incubation at a 10 μ M concentration resulted in rapid restoration of HIV-1 production (Table 1), indicating

* Corresponding author.

TABLE 1. Expression of HIV-1 proteins and surface CD4 in chronically infected CR10/N1T cells cultured in the presence of Ro 5-3335^a

Ro 5-3335 dose (μ M)	Cell viability (% living cells)	Expression of HIV-1 proteins		Surface CD4 expression (% positive cells)
		% IF-positive cells	p24 (ng/10 ⁶ cells)	
0	95	66	2,067	0.7
0.1	95	50	1,871	1.3
1	93	15	400	8.5
0.5	93	6	45	24.8
10	90	2	33	29.1
10 $\rightarrow$ 0	90	80	2,210	NT

^a Results of an experiment typical of five experiments performed with the indicated doses of Ro 5-3335 are shown. Cells were supplemented with the drug, HIV-1 antigens were detected, and surface CD4 was analyzed as described in the text. 10 $\rightarrow$ 0, CR10/N1T cells cultured for 6 days with 10 μ M Ro 5-3335 and then washed and cultured for 6 days in the absence of the drug. NT, not tested.

that the drug acts by reversibly suppressing HIV-1 expression and not by eliminating HIV-1-infected cells (14).

To determine the effect of Ro 5-3335 on the expression of major forms of HIV-1 RNA and on the levels of HIV-1 DNA, HIV-1-infected CR10/N1T cells were incubated with the drug for 6 days as described above. HIV-1 expression as monitored by the IF assay declined from 91% IF-positive cells for untreated cells (Fig. 1A) to 4% for drug-treated cells (Fig. 1B). To determine the steady-state levels of HIV-1 RNA, total cellular RNA was isolated by the guanidinium isothiocyanate method (5) and analyzed by Northern (RNA) blot hybridization (Fig. 1C). RNA samples (5 μ g) were electrophoresed in a 1% formaldehyde-agarose gel, transferred to a Nytran membrane (Schleicher and Schuell, Keene, N.H.), and hybridized by using the α -³²P-labeled 8.9-kb *SacI-SacI* fragment of the HIV-1-N1T-A clone DNA (25) as a probe, as previously described (25, 27). Duplicate

membranes were hybridized by using the α -³²P-labeled 1.2-kb *PstI* fragment from plasmid pK α -1 (7). The appearance of both full-length and spliced species of HIV-1 transcripts was significantly diminished in the presence of 5 μ M Ro 5-3335 (Fig. 1C). The levels of the tubulin mRNA remained the same in drug-treated and untreated cells (Fig. 1C), indicating that Ro 5-3335 has no indiscriminate effect on cellular gene transcription. These results extend our previous study using liquid RNA-RNA hybridization for HIV-1 RNA detection (14) by showing that the diminution of the production of viral proteins in the presence of Ro 5-3335 is due primarily to the inhibition of HIV-1 expression at the transcriptional level. This is the expected consequence of the HIV-1 Tat-antagonistic activity of Ro 5-3335, which has been shown to specifically inhibit Tat-TAR-mediated transactivation of the HIV-1 promoter in transient expression assays and in stable transfectants (14).

To evaluate the status of proviral DNA, total cellular DNAs from untreated and drug-treated CR10/N1T cells, shown in Fig. 1A and B, were analyzed by Southern blot hybridization (Fig. 1D). No difference either in relative levels of HIV-1 DNA or in the respective endonuclease digestion patterns (Fig. 1D) was found. That the viral DNA in Ro 5-3335-treated cells remained functional was shown in that removal of the drug allowed resumption of efficient expression of HIV-1 proteins within 6 days (Table 1). These results suggest that in chronically infected T cells in vitro (which carry mainly integrated proviral DNA [4]), Ro 5-3335 imposes a restriction on HIV-1 expression that approaches viral latency.

Restoration of surface CD4 display upon extinction of HIV-1 expression. In the experiment summarized in Table 1, the drug dose-dependent reduction in the synthesis of HIV-1 products in CR10/N1T cells engendered a reciprocal dose-dependent increase in surface CD4 display, from <1% CD4-bearing cells in the absence of Ro 5-3335 to 29% CD4-bearing cells after culture with 10 μ M drug. As expected, the reexpression of HIV-1 in CR10/N1T cells after removal of Ro 5-3335 (Table 1) diminished surface CD4 display to the levels observed in untreated HIV-1-infected cells (results not shown).

To investigate the mechanism of CD4 upregulation in the presence of the Tat antagonist, we analyzed Ro 5-3335-treated and control cells for the expression of CD4 mRNA and the levels of free CD4 55-kDa protein available for display on the cell surface (Fig. 2). Relative to the amounts of α -tubulin mRNA, the steady-state CD4 mRNA levels were comparable in HIV-1-infected and uninfected cells, regardless of the drug treatment and its effect on HIV-1 expression (Fig. 2A). This result suggested that the increase in CD4 display in the presence of Ro 5-3335 was not due to a drug-mediated augmentation of CD4 gene transcription. Next, we attempted to determine whether the Ro 5-3335-mediated extinction of HIV-1 expression increases the amount of free intracellular 55-kDa CD4 protein, that is, CD4 which is not bound to gp160 in intracellular complexes. Such complexes have been shown to accumulate in the endoplasmic reticulum, thus reducing the availability of CD4 for transport and insertion into the plasma membrane (1, 8, 15, 27). We performed immunoprecipitation studies using two anti-CD4 antibodies differing in their recognition of the gp120-CD4 binding site. In one experiment (Fig. 2B), we employed Leu3A, which competes with gp120 for the same binding site on CD4. Leu3A can precipitate intracellular CD4 only if it is not bound to the gp160 precursor protein (8, 12, 13, 16-18, 27). Control and drug-treated cells were

FIG. 1. Expression of HIV-1 antigens, RNA, and DNA in chronically infected CR10/N1T cells treated with the Tat antagonist Ro 5-3335. Chronically infected CR10/N1T cells (4) were treated with 5 μ M Ro 5-3335 for 6 days essentially as described in the text; the cells were then analyzed in parallel for HIV-1 (Fig. 1) and CD4 expression (Fig. 2). (A and B) Detection of HIV-1 antigens in untreated and treated cells, respectively, by IF. Magnification, ca. $\times$ 261. (C) Detection of HIV-1 RNA in Ro 5-3335-treated (+) and untreated (-) CR10/N1T cells by Northern blot hybridization. (D) HIV-1 DNA analysis in treated and untreated cells by Southern blot hybridization. A quantity of 20 μ g each of CR10/N1T DNA from drug-treated and untreated cells was digested with either *SstI* or *PvuII*, electrophoresed on a 1% agarose gel, and transferred to a Nytran membrane. The HIV-1 DNA probe and prehybridization and hybridization conditions were as described for RNA analysis (text and references 25 and 27). Lane M, molecular size markers.

FIG. 2. CD4 expression in Tat antagonist-treated and untreated HIV-1-infected CR10/N1T cells. Cells from the experiment whose results are shown in Fig. 1 were used. (A) CD4 mRNA levels. Five-microgram samples of RNA from control (–) and drug-treated (+) CR10 and CR10/N1T cells were analyzed as described previously (27). The panel shows double probing for CD4 mRNA and α -tubulin mRNA with an α - 32 P-labeled 3-kb *Eco*RI CD4 gene fragment from plasmid T4-pMV7 (16) and an α - 32 P-labeled 1.2-kb *Pst*I fragment from plasmid p κ -1 (7), respectively. (B) Detection of CD4 protein by radioimmunoprecipitation with Leu3A monoclonal antibody (see text for details). HTB 148 is a surface CD4-negative glial cell line (28). +, treated with Ro 5-3335; –, untreated. (C) Expression of surface CD4 as detected by flow cytometry. Actual fluorescence corrected for background is shown. Flow cytometry was performed as described in the text.

metabolically labeled for 6 h in the presence of 50 μ Ci each of [35 S]cysteine and [35 S]methionine per ml, and the cells were washed and lysed in a buffer containing 20 mM Tris-HCl (pH 8.0), 140 mM NaCl, 0.1 mM phenylmethylsulfonyl fluoride, 5 μ g of aprotinin per ml, 0.2 mM EGTA [ethylene glycol-bis(β -aminoethyl ether)-*N,N,N',N'*-tetraacetic acid], 0.2 mM sodium fluoride, 0.2% sodium deoxycholate, and 0.5% Nonidet P-40. Cell lysates were incubated with Leu3A bound to protein G-Sepharose (Pharmacia), and the conjugates were analyzed by electrophoresis on an 8% polyacrylamide gel (27). The Leu3A precipitate from Ro 5-3335-treated CR10/N1T cells contained clearly detectable 55-kDa CD4 protein (Fig. 2B), in accord with partial restoration of surface CD4 display in these cells as detected by flow cytometry (Fig. 2C). In contrast (and consistent with our previous study on CD4 downmodulation [27]), the Leu3A precipitate from extracts of untreated CR10/N1T cells contained a barely detectable 55-kDa band (Fig. 2B). This indicated a low level of free CD4 in these cells and coincided with the lack of CD4 display on the cell surface (Fig. 2C).

A more definitive demonstration of the role of intracellular CD4-gp160 complexes in both the diminution (in CR10/N1T cells) and restoration (in Tat antagonist-treated CR10/N1T cells) of surface CD4 expression was obtained by immunoprecipitation with T4-4, a rabbit polyclonal antiserum to CD4 which precipitates CD4-gp160 complexes (10). CR10/N1T and control CR10 cells were treated with Ro 5-3335 as described above; HIV-1 expression in CR10/N1T cells incubated in the presence of 10 μ M Ro 5-3335 declined from 92 to 2% IF-positive cells (Fig. 3, third and fourth lanes from left). Control and drug-treated cells were metabolically la-

FIG. 3. Effect of Tat antagonist on the levels of CD4-gp160 complexes in chronically HIV-1-infected cells. CR10/N1T and CR10 cells were untreated (–) or treated with the indicated doses of Ro 5-3335 for 6 days as described in the text. The cells were metabolically labeled, and immunoprecipitation was performed with the T4-4 antibody as described in the text and in the legend to Fig. 2. At bottom is shown the percentage of cells in each category staining positive for HIV-1 antigens by IF. Lane M, molecular size markers.

beled with 35 S-amino acids and lysed, and the cell lysates were used for immunoprecipitation with the T4-4 anti-CD4 antiserum (AIDS Research and Reference Reagent Program, Rockville, Md.) as described for Fig. 2 above. In extracts of untreated CR10/N1T cells, the T4-4 antibody coprecipitated CD4 and gp160 (Fig. 3, third lane from left), demonstrating the presence of CD4-gp160 complexes in these chronically HIV-1-infected cells. In contrast, undetectable amounts of labeled gp160 were coprecipitated in CR10/N1T cells treated with 10 μ M drug and a significant reduction in gp160 coprecipitation was observed in cells treated with an intermediate dose of the drug, which was partially effective in reducing HIV-1 expression (Fig. 3). Control rabbit serum did not precipitate any proteins in the 55- or 160-kDa regions (results not shown). These data indicate that the restoration of surface CD4 expression in Tat inhibitor-treated HIV-1-infected cells is due to the reduction in the levels of CD4-gp160 intracellular complexes, making CD4 available for display on the cell surface. It should be noted that the surface CD4 redisplay in the presence of Tat inhibitor did not attain the levels observed in uninfected cells (Table 1; Fig. 1). This can be attributed to an incomplete extinction of HIV-1 expression in chronically infected cells by Ro 5-3335 (Table 1; Fig. 1 and 3), as is also indicated by the drug dose dependence of the reduction in CD4-gp160 complex formation (Fig. 3).

We have shown that the reduction of HIV-1 expression in chronically infected, CD4-negative cells by treatment with a Tat antagonist permits partial redisplay of surface CD4 receptors. Our results indicate that this effect is due to a decrease in the amount of CD4 complexed with gp160 and a consequent elevation in the level of free (Leu3A-reactive) CD4. The Tat antagonist does not affect the steady-state levels of CD4 mRNA, indicating that the fluctuations in CD4 expression described here are posttranscriptional. Our data are consistent with previous reports that indicated a correlation among surface CD4 display, relative levels of HIV-1 expression, and formation of intracellular CD4-gp160 complexes (1, 3, 8, 12, 13, 15–17, 21, 27). In vivo, HIV-1 is most often found in CD4-positive T cells, and it has been postulated that most of these cells carry latent or poorly expressed

virus (22, 23, 26). It has been proposed that activation of these cells induces HIV-1 (2); this in turn is expected to downmodulate surface CD4 receptors (3). Inhibition of HIV-1 expression in virus-infected T cells by the Tat antagonist Ro 5-3335, as shown here, may not only prevent T-cell death but also restore the capacity of the cells to mediate immune responses.

We thank M. J. Potash for critical reading of the manuscript, M. M. Reddy and M. Grieco for help in flow cytometry, P. Maddon and R. Axel for providing the T4-pMV7 vector, and N. J. Cowan for the p α -1 vector.

This work was supported in part by grant AI 27397 for a National Cooperative Drug Discovery Group from NIH-NIAID and by a grant from the Pediatric AIDS Foundation.

REFERENCES

- Bour, S., F. Boulterice, and M. A. Wainberg. 1991. Inhibition of gp160 and CD4 maturation in U937 cells after both defective and productive infections by human immunodeficiency virus type 1. *J. Virol.* **65**:6387-6396.
- Bukrinsky, M. I., T. L. Stanwick, M. P. Dempsey, and M. Stevenson. 1991. Quiescent T lymphocytes as an inducible virus reservoir in HIV-1 infection. *Science* **254**:423-427.
- Butera, S. T., V. L. Perez, B.-Y. Wu, G. J. Nabel, and T. M. Folks. 1991. Oscillation of the human immunodeficiency virus surface receptor is regulated by the state of viral activation in a CD4⁺ cell model of chronic infection. *J. Virol.* **65**:4645-4653.
- Casareale, D., M. Stevenson, K. Sakai, and D. J. Volsky. 1987. A human T-cell line resistant to cytopathic effects of the human immunodeficiency virus (HIV). *Virology* **156**:40-49.
- Chirgwin, J. J., A. E. Przybyla, R. J. MacDonald, and W. J. Rutter. 1979. Isolation of biologically active ribonucleic acid from sources enriched in ribonuclease. *Biochemistry* **18**:5294-5299.
- Clark, S., W. Jefferies, N. Barclay, J. Gagnon, and A. Williams. 1987. Peptide and nucleotide sequences of rat HIV-1 (W3/25) antigen: evidence for derivation from a structure with four immunoglobulin-related domains. *Proc. Natl. Acad. Sci. USA* **84**:1649-1653.
- Cowan, N. J., P. R. Dobner, E. V. Fuchs, and D. W. Cleveland. 1983. Expression of human α -tubulin genes: interspecies conservation of 3' untranslated regions. *Mol. Cell. Biol.* **3**:1738-1745.
- Crise, B., L. Buonocore, and J. K. Rose. 1990. CD4 is retained in the endoplasmic reticulum by the human immunodeficiency virus type 1 glycoprotein precursor. *J. Virol.* **64**:5585-5593.
- Dalgleish, A. G., P. C. Beverly, P. R. Clapham, D. H. Crawford, M. F. Greaves, and R. A. Weiss. 1984. The CD4 (T4) antigen is an essential component of the receptor for AIDS retrovirus. *Nature (London)* **312**:763-767.
- Deen, K. C., J. S. McDougal, R. Inacker, G. Golena-Wasserman, J. Arthos, J. Rosenberg, P. J. Maddon, R. Axel, and R. W. Sweet. 1988. A soluble form of CD4 (T4) protein inhibits AIDS virus infection. *Nature (London)* **331**:82-84.
- Geleziunas, R., S. Bour, F. Boulterice, J. Hiscott, and M. A. Wainberg. 1991. Diminution of CD4 surface protein but not CD4 messenger RNA levels in monocytic cells infected by HIV-1. *AIDS* **5**:29-33.
- Hoxie, J. A., J. D. Alpers, J. L. Rackowski, K. Hubner, Haggarty, B. S., A. J. Cedarbaum, and J. C. Reed. 1986. Alterations in T4 (CD4) protein and mRNA synthesis in cells infected with HIV. *Science* **234**:1123-1127.
- Hoxie, J. A., B. S. Haggarty, J. L. Rackowski, N. Pillsbury, and J. A. Levy. 1985. Persistent noncytopathic infection of normal human T lymphocytes with AIDS-associated retrovirus. *Science* **229**:1400-1402.
- Hsu, M.-C., A. D. Schutt, M. Holly, L. W. Slice, M. I. Sherman, D. D. Richman, M. J. Potash, and D. J. Volsky. 1991. Inhibition of HIV replication in acute and chronic infections *in vitro* by a Tat antagonist. *Science* **254**:1799-1802.
- Jabbar, M. A., and D. P. Nayak. 1990. Intracellular interaction of human immunodeficiency virus type 1 (ARV-2) envelope glycoprotein gp160 with CD4 blocks the movement and maturation of CD4 to the plasma membrane. *J. Virol.* **64**:6297-6304.
- Kawamura, I., Y. Koga, N. Oh-Hori, K. Onodera, G. Kimura, and K. Nomoto. 1989. Depletion of the surface CD4 molecule by the envelope protein of human immunodeficiency virus expressed in a human CD4⁺ monocytoid cell line. *J. Virol.* **63**:3748-3754.
- Kazazi, F., J.-M. Mathijs, P. Foley, and A. L. Cunningham. 1989. Variations in CD4 expression by human monocytes and macrophages and their relationship to infection with the human immunodeficiency virus. *J. Gen. Virol.* **70**:2661-2672.
- Klatzmann, D., F. Barre-Sinoussi, M. T. Nugeyre, C. Dauguet, E. Vilmer, C. Griscelli, F. Brun-Vazinet, C. Rouzioux, J. C. Gluckman, J. C. Chermann, and L. Montagnier. 1984. Selective tropism of lymphadenopathy associated virus (LAV) for helper-inducer T lymphocytes. *Science* **225**:59-63.
- Maddon, P. J., A. G. Dalgleish, J. S. McDougal, P. R. Clapham, R. A. Weiss, and R. Axel. 1986. The T4 gene encodes the AIDS virus receptor and is expressed in the immune system and the brain. *Cell* **47**:333-348.
- Maddon, P. J., D. R. Littman, M. Godfrey, D. E. Maddon, L. Chess, and R. Axel. 1985. The isolation and nucleotide sequence of a cDNA encoding the T cell surface protein T4: a new member of the immunoglobulin gene family. *Cell* **42**:93-104.
- McDougal, J. S., A. Mawle, A. P. Cort, J. K. Nicholson, G. D. Cross, J. A. Scheppeler-Campbell, D. Hicks, and J. Sligh. 1985. Cellular tropism of the human retrovirus HTLV-III/LAV. I. Role of T cell activation and expression of T4 antigen. *J. Immunol.* **135**:3151-3162.
- Pantaleo, G., C. Graziosi, L. Butini, P. A. Pizzo, S. M. Schnittman, D. P. Kotler, and A. S. Fauci. 1991. Lymphoid organs function as major reservoirs for human immunodeficiency virus. *Proc. Natl. Acad. Sci. USA* **88**:9838-9842.
- Psallidopoulos, M. C., S. M. Schnittman, L. M. Thompson III, M. Baseler, A. S. Fauci, H. C. Lane, and N. P. Salzman. 1989. Integrated proviral human immunodeficiency virus type 1 is present in CD4⁺ peripheral blood lymphocytes in healthy seropositive individuals. *J. Virol.* **63**:4626-4631.
- Rosenberg, Z. F., and A. S. Fauci. 1991. Immunopathogenesis of HIV infection. *FASEB J.* **5**:2382-2390.
- Sakai, K., S. Dewhurst, X. Ma, and D. J. Volsky. 1988. Differences in cytopathogenicity and host cell range among infectious molecular clones of human immunodeficiency virus type 1 simultaneously isolated from an individual. *J. Virol.* **62**:4078-4085.
- Schnittman, S. M., M. C. Psallidopoulos, H. C. Lane, L. Thompson, M. Boseler, F. Massari, C. H. Fox, N. P. Salzman, and A. S. Fauci. 1989. The reservoir for HIV-1 in human peripheral blood is in a T cell that maintains expression of CD4. *Science* **245**:305-308.
- Stevenson, M., X. Zhang, and D. J. Volsky. 1987. Downregulation of cell surface molecules during noncytopathic infection of T cells with human immunodeficiency virus. *J. Virol.* **61**:3741-3748.
- Volsky, B., K. Sakai, M. M. Reddy, and D. J. Volsky. 1992. A system for high efficiency replication of HIV-1 in neural cells and its application to anti-viral evaluation. *Virology* **186**:303-308.
- Volsky, D. J., M. G. Pellegrino, G. Li, K. A. Logan, J. E. Aswell, N. P. Lawrence, and S. R. Decker. 1990. Titration of human immunodeficiency virus type 1 (HIV-1) and quantitative analysis of virus expression *in vitro* using liquid RNA-RNA hybridization. *J. Virol. Methods* **28**:257-272.