

Interleukin-7 Induces Expression of Latent Human Immunodeficiency Virus Type 1 with Minimal Effects on T-Cell Phenotype

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Latent human immunodeficiency virus type 1 (HIV-1) persists even in patients treated with antiretroviral therapy. New treatment strategies are therefore needed to eradicate this latent viral reservoir without reducing immune cell function. We characterize the interleukin-7 (IL-7)-induced stimulation of primary human T cells and thymocytes and demonstrate, using the SCID-hu model, that IL-7 induces substantial expression of latent HIV while having minimal effects on the cell phenotype. Thus, IL-7 is a viable candidate to activate expression of latent HIV and may facilitate immune clearance of latently infected cells.

Latent human immunodeficiency virus (HIV) has been documented in both memory and naive T lymphocytes (9, 12, 29, 32, 35), and it is estimated to require up to 60 years to eliminate this reservoir through the use of highly active antiretroviral therapy (HAART) alone (17, 48). Thus, new treatment strategies designed to purge the pool of latently infected cells are needed.

The cell activation state plays a critical role in the ability of HIV type 1 (HIV-1) to achieve and maintain active replication (26). Cytokine treatment, which can alter the activation state of the host cell and thus alter patterns of viral infection and replication (42, 43), has been previously proposed as an adjunctive therapy both to activate the reservoir of latently infected T cells and to improve immune function in immunodeficient patients. A combination of interleukin-6 (IL-6), IL-2, and tumor necrosis factor alpha (TNF- α) induces emergence from latency *in vitro* (7, 10), and simultaneous treatment with IL-2 and HAART can transiently increase CD4 T cells and reduce the pool of resting cells containing replication-competent HIV in patients (11). Naive T cells treated with these cytokines become activated, however, and some cells lose naive phenotype and behavior (44, 45), which could impact their ability to mount an immune response. Previous studies showed that IL-7, in addition to stimulating T cells (13, 39, 45) and thymocytes (8, 31, 42), allows productive HIV infection of T cells while having minimal effects on naive and memory phenotypes (39, 45), prompting us to assess IL-7 as a therapeutic agent to induce activation of latent HIV-1 and to define its action on quiescent human cell populations known to harbor latent virus.

Effect of IL-7 on mature thymocytes. The SCID-hu HIV latency model produces high levels of latently infected mature human thymocytes and peripheral naive T cells due to the decrease in cellular gene transcription during thymopoiesis (7). To precisely characterize the effects of IL-7 on cell subsets relevant to latent populations amenable to study in the

SCID-hu model, 4-day whole-lobe human fetal thymic organ cultures (3, 6, 37) in serum-free medium (23, 41) were used. In our initial experiments, we monitored thymocytes for the effect of IL-7 on expression of the developmental and activation markers CD69, CD25, and CD45RA and on the IL-7 receptor α subunit, CD127. Other than the expected down regulation of CD127, we detected up regulation of CD69, a marker of positive selection, and increases in CD45RA, but only minimal change in CD25 (not shown).

A four-color flow cytometric cell cycle assay (7, 38) was used to simultaneously examine two surface markers and cellular DNA and RNA synthesis, allowing identification of cells in different stages of the cell cycle (Fig. 1A). Both CD4⁺ CD8⁻ (CD4SP) and CD8⁺ CD4⁻ (CD8SP) thymocytes displayed increased percentages of cells in the G_{1B} and S stages of the cell cycle when cultured with IL-7, increasing the percentage of activated thymocytes an average of 1.8-fold \pm 0.2-fold (CD4SP) or 3.7-fold \pm 1.7-fold (CD8SP) (Fig. 1B). These increases are consistent with recently reported experiments in which IL-7 increased Ki-67 staining within these subsets in human fetal thymic organ cultures (31). IL-7 induced increases in production of cellular RNA in mature thymocyte subsets (Fig. 1B), strongly suggesting to us that this cytokine should be capable of activating the expression of latent HIV-1 within the same cell subsets derived from the SCID-hu model.

IL-7 induces expression of latent HIV-1. To determine whether IL-7 can force HIV-1 out of the latent state, SCID-hu mice (1, 5) were infected by intra-implant injection (7) with HIV strain JR-CSF (CCR5 tropic) or NL-r-HSAS (24) (a CXCR4-tropic strain bearing murine CD24 reporter sequences). At 4.5, 7, or 9 weeks postinfection, thymocytes or peripheral cells from infected or mock-infected mice were cultured in the presence of antiretroviral agents, as described previously (7), and then stimulated with anti-CD3 and anti-CD28 antibodies, with anti-CD3 antibodies alone, with IL-2, or with IL-7 for 3 days *in vitro*. IL-7 stimulated substantial p24^{gag} production (as determined by enzyme-linked immunosorbent assay of supernatants) both from mature thymocytes and from pooled peripheral cells and, moreover, consistently induced higher p24 levels than did either anti-CD3 antibodies alone or IL-2 (Fig. 2A). Three days following stimulation, IL-7 induced

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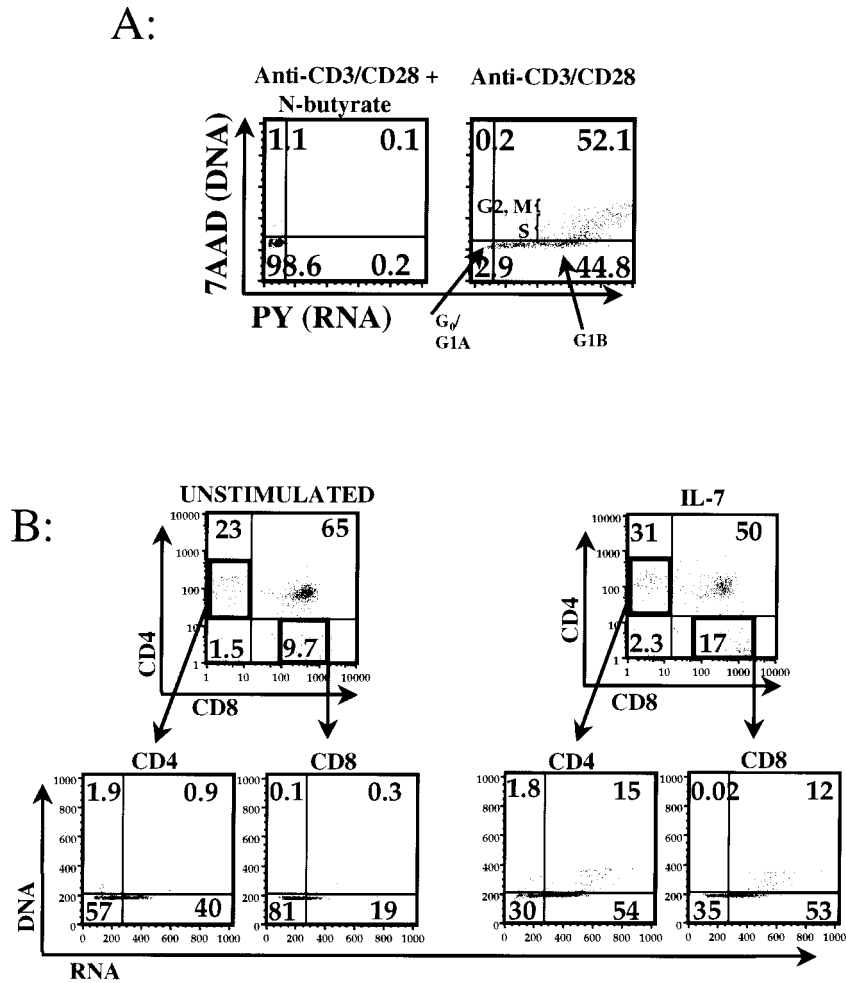


FIG. 1. Effect of IL-7 on human thymocytes. (A) Anti-CD3, anti-CD28 costimulated normal peripheral blood lymphocytes, either alone (right panel) or treated with N-butyrated (left panel), are shown with the cell cycle status indicated in each quadrant, as an example of the assay used in panel B, and throughout this work. DNA is depicted on the vertical axis, and RNA is depicted on the horizontal axis. The position of the vertical axis marks the division between the G_{1A} and G_{1B} stages of the cell cycle and is determined by the N-butyrated-treated cells, which are blocked at the G_{1A}-to-G_{1B} transition. Positions indicating stages in the cell cycle are illustrated in the right panel. (B) Four-color cell cycle analysis was performed on fetal thymocytes in whole-lobe thymic organ culture either alone or in the presence of IL-7 for 4 days. Three independent experiments were performed, of which one representative is shown. Top row, CD4SP and CD8SP cell populations were backgated (arrows) and analyzed for the presence of IL-7-induced changes in cell cycle progression (bottom row).

more virus-encoded reporter gene (mu CD24) expression from latently infected cells from NL-r-HSAS-infected animals than did IL-2 and was nearly as effective as costimulation through CD3 and CD28 (Fig. 2B). The magnitude of reporter expression with IL-7 stimulation was also increased over that of IL-2 (Fig. 2B). IL-7 is thus the most active cytokine in terms of stimulation of latent virus that we have found to date (7).

Effect of IL-7 on quiescent human peripheral blood T lymphocytes. Our finding that IL-7 induced expression of latent virus from peripheral blood and splenocytes in the SCID-hu model suggested that this cytokine would be effective in purging latent virus from peripheral T lymphocytes in patients. This may only be clinically relevant, however, if quiescent cells targeted by the cytokine are able to maintain functional phenotype. This was assessed by monitoring various activation and maturation markers. Quiescent human lymphocytes were purified from normal human peripheral blood and depleted of

cells expressing HLA-DR, CD25, CD69, CD14, and/or CD19 as described previously (26). In some experiments, either CD4 or CD8, in combination with either CD45RA or CD45RO, was also negatively selected to generated four quiescent populations: CD45RA⁻/CD4⁻, CD45RA⁻/CD8⁻, CD45RO⁻/CD4⁻, and CD45RO⁻/CD8⁻. Cells were cultured for 3 to 6 days in medium alone, stimulated with anti-CD3, costimulated with anti-CD3 and anti-CD28, or treated with 10 ng of IL-7/ml and assessed for changes in expression of activation markers and for effects on RNA and DNA synthesis.

Both CD45RO⁻ and CD45RA⁻ cells displayed increased levels of CD25 in the presence of IL-7, although increased expression of CD69, an early marker of activation, was not seen. CD27 was up regulated by both IL-2 and IL-7 but not by costimulation (not shown). Although we observed a slight increase in the percentage of CD45RA⁻ and CD45RO⁻ quiescent cells that expressed CD45RA and in the mean fluores-

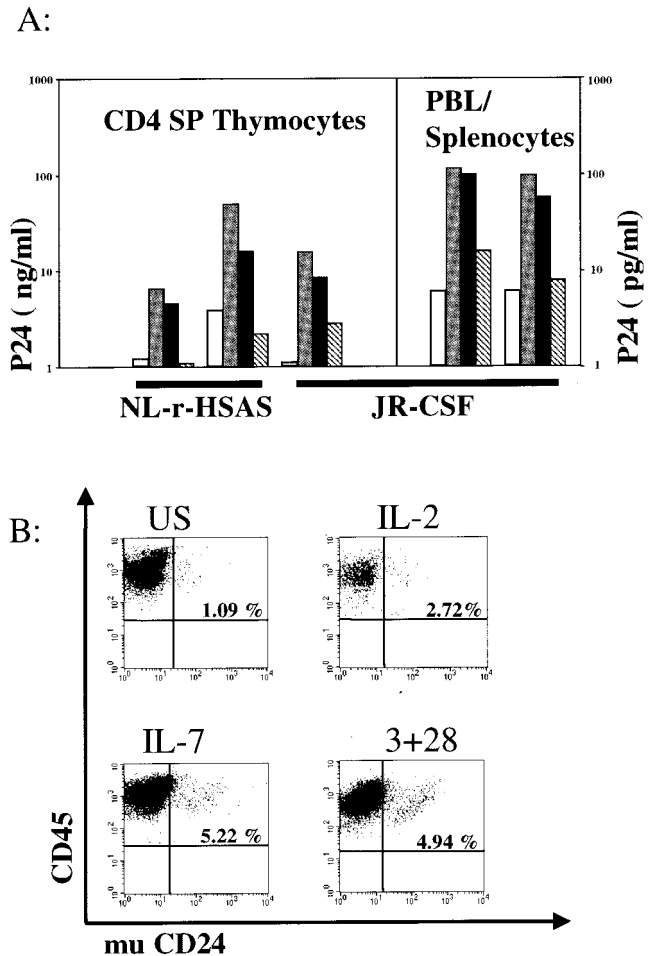


FIG. 2. Effect of IL-7 on HIV-1 latency. (A) CD4SP thymocytes or pooled peripheral blood lymphocytes (PBL) and splenocytes were isolated from infected (HIV_{NL-r-HSAS} or HIV_{JR-CSF}) SCID-hu Thy/Liv implants and were cultured for three days in the presence of Protease Inhibitor, and under the following stimulation conditions: unstimulated (white), costimulated (grey), IL-7 (black), and IL-2 (striped). HIV-1 p24^{gag} protein concentrations in the supernatants were measured to assess virus production. Each grouping of four bars represents one experiment. Unstimulated peripheral cells fell below the limit of detection (6 pg/ml). (B) Expression of virus-encoded reporter (mu CD24) indicating activation of latent virus. Mature CD4 SP thymocytes from NL-r-HSAs infected implants were treated for 3 days without stimulation (US) or with IL-2, IL-7, or anti-CD3/CD28 costimulation as indicated. Quadrant gates were set on isotype controls.

cence intensity for this marker, we observed minimal changes in the expression of CD45RO (Fig. 3A), CD4, and CD8 (not shown). The IL-7 receptor was down regulated in all subsets treated with IL-7, suggesting that binding and internalization of the cytokine was occurring (not shown).

Significantly, when stimulated with IL-7 or IL-2, CCR7 and CD62L remained substantially unchanged in memory (CD45RA⁻) subsets, although CCR7 was slightly up regulated in the CD45RO⁻/CD4⁻ subset and slightly down regulated in the CD45RO⁻/CD8⁻ subset (Fig. 3B). In contrast, both CD62L and CCR7 were dramatically down regulated by costimulation (Fig. 3B). Thus, in general, the overall expression

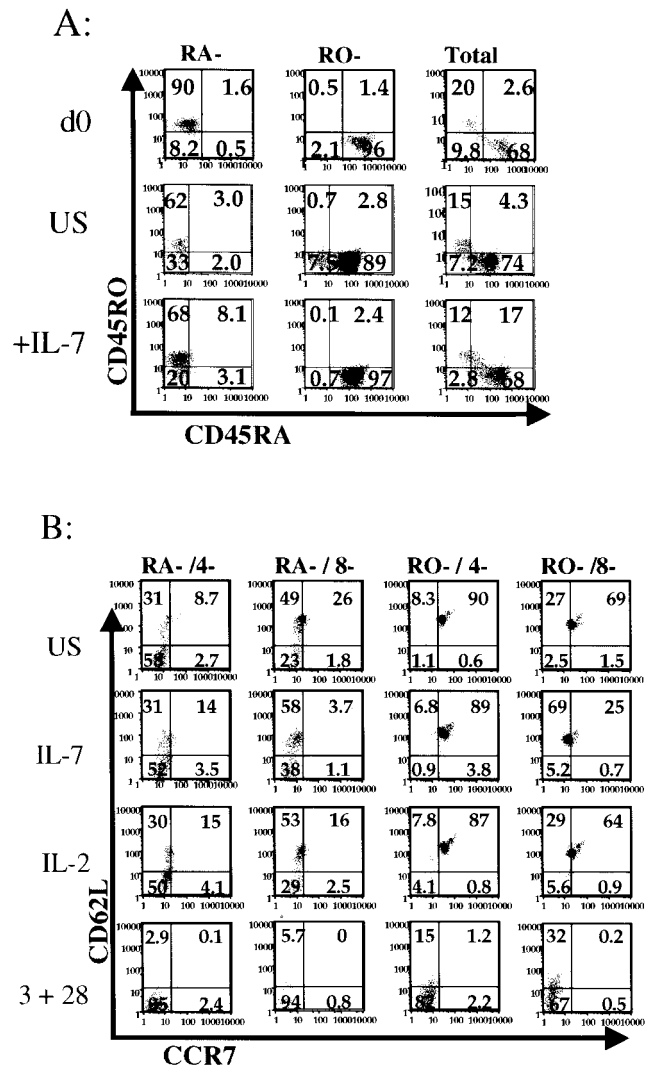


FIG. 3. Effect of IL-7 on surface phenotype of quiescent human lymphocytes. Total, CD45RA⁻, or CD45RO⁻ quiescent lymphocytes were cultured in the presence or absence of IL-7. Developmental and stimulation markers were examined using flow cytometric methods. (A) Total, CD45RA⁻, or CD45RO⁻ quiescent lymphocytes were cultured for 4 days as described previously and then analyzed for expression of CD45RA (x axis) and CD45RO (y axis) in the presence (bottom row) and absence (middle row) of IL-7, as well as on day 0 (top row). (B) CD45RA⁻/4⁻, CD45RA⁻/8⁻, CD45RO⁻/4⁻, and CD45RO⁻/8⁻ quiescent lymphocytes were isolated and cultured under the following stimulation conditions: unstimulated, IL-7, IL-2, or anti-CD3 and anti-CD28 costimulation for 3 days. Lymphocyte subsets were then analyzed for changes in expression of CCR7 (x axis) and CD62-L (y axis).

profile in these highly purified quiescent lymphocyte subsets remained stable when they were stimulated with IL-7.

Although treatment with IL-7 does not appear to negatively perturb the phenotype of the quiescent populations described above, elimination of the viral reservoir in the periphery may be achievable only if quiescent peripheral lymphocytes are capable of responding sufficiently to the stimulatory effects of IL-7. We therefore tested the ability of this cytokine to induce cell cycle progression in these same four subpopulations. IL-7, in contrast to IL-2, increased cellular RNA expression and

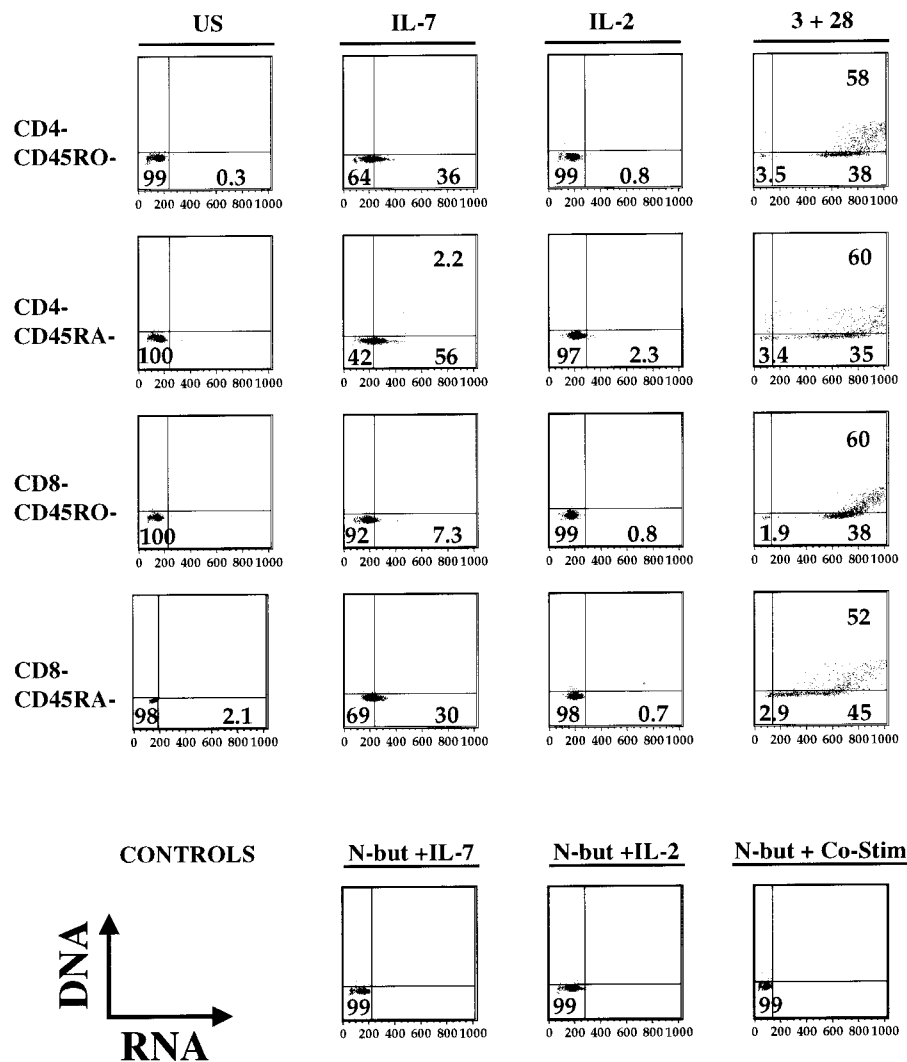


FIG. 4. Effect of IL-7 on cell cycle status of quiescent human lymphocytes. Quiescent CD45RA⁻/CD4⁻, CD45RA⁻/CD8⁻, CD45RO⁻/CD4⁻, and CD45RO⁻/CD8⁻ lymphocytes were isolated, and cultured under the following stimulation conditions: unstimulated, IL-7, IL-2, or anti-CD3 and anti-CD28 costimulation. After 3 days, cells were stained to detect DNA (y axis) and RNA (x axis) and analyzed to determine cell cycle status. Control stimulations performed in the presence of cell cycle inhibitor (N-acetyl butyric acid) and were used to set quadrant positions (bottom row). Seven similar experiments were performed.

entry into G_{1B} in all subpopulations analyzed (Fig. 4). The observed increase in RNA is maintained for at least 15 days in culture (not shown).

IL-7 is required for thymocyte development (34), enhances T-cell function and survival, (2, 4, 25), and has been reported to induce proliferation of naive CD45RA⁺ cell populations in peripheral blood while maintaining their naive phenotype (21, 39, 45). Our finding that IL-7 increased both RNA expression and DNA synthesis in mature thymocytes and induced RNA expression in stringently purified, quiescent CD4 and CD8 as well as CD45RA⁺ and CD45RO⁺ T cells is consistent with previously reported results for murine (46) and human (13, 15, 42) cells and with IL-7's ability to induce latent virus expression in the SCID-hu system.

Consistent with previous data (21, 45, 47), IL-7 altered cell surface expression of a limited set of activation antigens on quiescent T cells, most dramatically the IL-2 receptor. Further,

IL-7 induced only minimal perturbations in the expression of the naive homing and adhesion molecules CCR7 and CD62L, suggesting that IL-7 treatment would not impair cell migration patterns or behavior. Together, our data point to an IL-7-induced activation state distinct from that produced by costimulation, characterized by retention of phenotypic and functional markers and progression to the early G_{1B} stage of the cell cycle.

While our results in the SCID-hu model directly pertain to activation of latent virus in the naive quiescent cell subset, our cell cycle analysis of memory cells suggests that latent virus in this subset would behave similarly. It remains unclear whether IL-7 activation of latent virus occurs through a direct effect on the long terminal repeat. Signaling through the IL-7 receptor occurs through activation of Janus family kinases (JAK1/3), which then recruit signal transducers and activators of transcription (STAT) family members (STAT1, STAT3, and

STAT5). In addition, IL-7 can activate the PI3 kinase cascade via an interaction with STAT3 (33). Very recent studies suggest that only the STAT5-mediated IL-7 signaling pathway is involved in IL-7-induced effects on HIV expression during de novo infection (15). Activation of the latent reservoir may behave similarly.

Any potential immunotherapy must be considered for its effects on immune function, on viral pathogenesis, and on latently infected cells. Although it is thought that any increase in viral production associated with cytokine stimulation would be mitigated by HAART, several reports show that cytokines can increase HIV replication (16, 42). Recent work has demonstrated a correlation between CD4-T-cell depletion and increased circulating levels of IL-7 (20, 30). This correlation is important in light of IL-7's emerging role as a regulator of T cell homeostasis, both through peripheral expansion (19, 40) and through thymus-dependent mechanisms (28). IL-7 has also been linked to emergence of syncytium-inducing isolates during end-stage HIV disease (27). Further, increased levels of IL-7 are seen in some autoimmune diseases (14, 22), and chronic elevation of IL-7 in animal models has led to lymphoproliferative disorders (18, 36). Thus, a more detailed understanding of the *in vivo* effects of cytokine stimulation under conditions of HAART and HIV infection is warranted.

Our results indicate that IL-7 has minimal effects on T-cell phenotype and suggest that this cytokine is an excellent candidate for further study as an adjunctive therapy designed to purge the pool of latently infected T cells.

We thank G. Bristol, R. Cortado, and A. Kacena for valuable technical assistance and B. Jamieson for helpful discussions.

This work was supported by NIH grants AI36059 and AI36554, the UCLA CFAR, and the James B. Pendleton Charitable Trust. D.D.S.-A. was supported by a UCLA AIDS Institute fellowship. Y.D.K. was supported by NIDCR grant T32DF0796.

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