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HIV Envelope Induces Virus Expression from Resting CD4\(^+\) T Cells Isolated from HIV-Infected Individuals in the Absence of Markers of Cellular Activation or Apoptosis

Audrey L. Kinter, \(^2\) Craig A. Umscheid, \(^*\) James Arthos, \(^*\) Claudia Cicala, \(^*\) Yin Lin, \(^*\) Robert Jackson, \(^*\) Eileen Donoghue, \(^*\) Linda Ehler, \(^*\) Joseph Adelsberger, \(^\dagger\) Ronald L. Rabin, \(^\ddagger\) and Anthony S. Fauci \(^*\)

Resting CD4\(^+\) T cells containing integrated HIV provirus constitute one of the long-lived cellular reservoirs of HIV in vivo. This cellular reservoir of HIV had been thought to be quiescent with regard to virus replication based on the premise that HIV production in T cells is inexorably linked to cellular activation as determined by classical activation markers. The transition of T cells within this HIV reservoir from a resting state to an activated HIV-producing state is believed to be associated with a shortened life span due to susceptibility to activation-associated apoptosis. Evidence is mounting, however, that HIV production may occur in T cells that have not undergone classic T cell activation. HIV encodes several proteins, including envelope and Nef, which trigger a variety of signaling pathways associated with cellular activation, thereby facilitating HIV replication in nondividing cells. The present study demonstrates that production of infectious virus from resting CD4\(^+\) T cells isolated from HIV-infected individuals can be induced following exposure of these cells to HIV-1 recombinant (oligomeric gp140) envelope protein. Envelope-mediated induction of HIV expression occurs in the presence of reverse transcriptase inhibitors and is not associated with markers of classic T cell activation, proliferation, or apoptosis. The ability of HIV envelope to induce virus replication in HIV-infected resting CD4\(^+\) T cells without triggering apoptosis provides a mechanism for the virus itself to directly participate in the maintenance of HIV production from this cellular reservoir. *The Journal of Immunology, 2003, 170: 2449–2455.*

Chronically HIV-infected resting CD4\(^+\) T cells constitute a major long-lived viral reservoir (1; reviewed in Ref. 2) that poses a significant obstacle to the ultimate eradication of HIV in infected individuals (3–5). Although virus production has not been demonstrated in freshly isolated, HIV-infected resting CD4\(^+\) T cells, infectious virus can be detected in vitro following stimulation of these cells with T cell-activating agents (3–7). Similarly, HIV production in vivo has been thought to require the transition of these cells from a resting to an activated state (reviewed in Ref. 2), an event that presumably shortens their life span due to increased susceptibility to activation-associated apoptosis (8, 9) and perhaps to virus-mediated cytopathic effects (9–11) and immune-mediated cytolysis (12). Evidence is accumulating, however, that productive HIV infection in T cells is not invariably linked to T cell activation. Previous studies have demonstrated that under certain in vitro conditions, nondividing T cells can support HIV replication (13–15). In addition, recent analyses of cell-associated HIV RNA in HIV-infected individuals, either early in disease (16) or following long-term highly active antiretroviral therapy (17), demonstrate that HIV RNA is produced, albeit at low levels, in peripheral blood or lymph node-derived resting CD4\(^+\) T cells in vivo. These data support the concept that HIV replication may occur within a CD4\(^+\) T cell that is activated below the threshold required for the expression of classical T cell activation markers and for the progression to proliferation.

Previous studies have demonstrated that HIV encodes for numerous regulatory proteins that interact with cellular activation pathways, thereby facilitating HIV replication following entry of virus into nondividing cells (reviewed in Refs. 18 and 19). Similarly, the interaction between CD4\(^+\) cells and HIV envelope protein (Refs. 21–27; reviewed in Ref. 28) or inactivated virions (24, 29, 30) activates a large variety of cellular gene products associated with cellular activation and in this way may prime nondividing cells for the ability to support subsequent HIV infection. Counteracting these potentially positive effects on HIV replication, HIV envelope has also been demonstrated to enhance apoptosis in activated CD4\(^+\) T cells resulting, ultimately, in a decrease in the output of virions from these cells (Refs. 9, 30–33; reviewed in Refs. 28 and 34). Therefore, the net effect of the interaction between HIV envelope and CD4\(^+\) T cells on HIV replication may depend on the activation state of the cell and subsequently on the balance between the HIV-inducing and the cell death-inducing signals generated.

In contrast to the effects of most HIV regulatory proteins (reviewed in Refs. 18 and 19), HIV envelope-mediated effects are not dependent on infection of target cells, but, rather, could be triggered by soluble protein or defective virions. In this regard, HIV proteins (35) and RNA (36) have been detected in lymphoid tissue for months to years after initiation of highly active antiretroviral therapy (17), demonstrate that HIV RNA is produced, albeit at low levels, in peripheral blood or lymph node-derived resting CD4\(^+\) T cells in vivo. These data support the concept that HIV replication may occur within a CD4\(^+\) T cell that is activated below the threshold required for the expression of classical T cell activation markers and for the progression to proliferation.

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\(^2\) Address correspondence and reprint requests to Dr. Audrey Kinter, Laboratory of Immunoregulation, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Building 10, Room 6A17, 10 Center Drive, MSC-1576, Bethesda, MD 20892-1576. E-mail address: akinter@niaid.nih.gov

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therapy and suppression of plasma viremia to <50 copies/mL. In addition, HIV envelope protein or gp120-Ab complexes have been detected in the sera (37), in tissue (38) and on the surface of CD4+ T cells and monocytes (39) of HIV-infected individuals.

The present study investigates the effects of recombinant HIV-1 oligomeric envelope glycoprotein (rEnv) on endogenous HIV expression and cellular activation in resting (CD25−, HLA-DR−, CD69−, nondividing) CD4+ T cells isolated from HIV-infected individuals. Oligomeric rEnv, derived from both X4 and R5 HIV strains, was found to induce the expression of HIV from purified resting CD4+ T cells isolated from the majority of infected individuals. HIV expression from resting CD4+ T cells could be detected in vitro in the presence of antiretroviral drugs. Furthermore, envelope-mediated induction of infectious HIV expression was not associated with classical markers of T cell activation, progression through the cell cycle, or apoptosis, although certain early cellular signaling events could be detected. These data demonstrate that the net effect of the interaction between envelope and resting CD4+ T cells favors HIV production and underscores the potential contribution of this long-lived viral reservoir to ongoing low level viral replication in vivo.

Materials and Methods

Generation and purification of oligomeric HIV-1 rEnv

The anchor domain-deleted (gp140) oligomeric HIV-1 recombinant envelope glycoprotein (rEnv) was prepared and purified as previously described (22, 40). All protein preparations were tested for endotoxin by the Limulus amebocyte lysate method (BioWhittaker, Walkersville, MD); only preparations with undetectable levels of endotoxin were used. The m.w. of the protein preparation was estimated by gel filtration chromatography and exhibited a range consistent with primarily trimeric, but also mono- and dimeric, proteins.

Isolation and culture of resting CD4+ T cells from HIV-infected donors

Apheresis (National Institutes of Health Protocol 81-1-164) was performed on HIV-infected donors who were at various stages of HIV disease (CD4+ T cell range, 200-1000; viral load range, <50–200,000 copies/ml). PBMC, obtained by Ficoll-Hypaque density gradient centrifugation, were exposed to a cocktail of immunomagnetic beads (Stem Cell Technologies, Vancouver, Canada) to obtain resting (CD25−, CD69−, HLA-DR−) CD4+ T cells by negative selection. CD4+ T cells were typically 97−99% pure, and cells expressing activation Ags represented <1−2% of this population. Resting CD4+ T cells were resuspended in medium (Life Technologies, Gaithersburg, MD; RPMI 1640 supplemented with 10% FBS, 1 mM antibiotics, 1 mM glutamine, and 1 mM HEPES buffer) at 10−25 million cells/ml, and 200 μl of cell suspension was placed onto the surface of a 24-well tissue culture plate. Cells were exposed to various concentrations (1−100 nM) of rEnv – that the net effect of the interaction between envelope and resting CD4+ T cells and monocytes (39) of HIV-infected individuals.

The present study investigates the effects of recombinant HIV-1 oligomeric envelope glycoprotein (rEnv) on endogenous HIV expression and cellular activation in resting (CD25−, HLA-DR−, CD69−, nondividing) CD4+ T cells isolated from HIV-infected individuals. Oligomeric rEnv, derived from both X4 and R5 HIV strains, was found to induce the expression of HIV from purified resting CD4+ T cells isolated from the majority of infected individuals. HIV expression from resting CD4+ T cells could be detected in vitro in the presence of antiretroviral drugs. Furthermore, envelope-mediated induction of infectious HIV expression was not associated with classical markers of T cell activation, progression through the cell cycle, or apoptosis, although certain early cellular signaling events could be detected. These data demonstrate that the net effect of the interaction between envelope and resting CD4+ T cells favors HIV production and underscores the potential contribution of this long-lived viral reservoir to ongoing low level viral replication in vivo.

Flow cytometric analysis of cellular activation, cell cycle, and apoptosis

Resting CD4+ T cells were harvested at various time points (2 h, 6 h, 24 h, 2 days, 5 days, 8 days, and 10 days) following treatment with either oligomeric rEnv (10−100 nM) or PHA. Analysis of apoptosis was conducted as previously described (42) by staining for annexin V (BD PharMingen, San Diego, CA; in propidium iodide (PI, BD PharMingen) exclusion gate) on cells harvested at 2 h, 6 h, 24 h, and 2−7 days. Cell cycle analysis (PI incorporation into DNA of permeabilized cells) and expression of surface activation markers (CD25, CD69, HLA-DR; BD PharMingen) was assessed on cells harvested at 24 h and 2, 5, 8, and 10 days using standard staining techniques.

Analysis of the effect of oligomeric rEnv on the phosphorylation of FAK and ZAP-70 in resting CD4+ T cells

Ten million resting CD4+ T cells were resuspended in fresh RPMI 1640 medium containing 1% albumin and 10 mM HEPES, pH 7.4, and were treated, at a ratio of five beads per cell, with goat anti-mouse Ab immunomagnetic beads (DynaBiotek, Lake Success, NY) conjugated to mouse anti-gp41 Ab (clone 3D6; AIDS Reagent Repository, Rockville, MD) that had been preincubated with 1.2 μg/ml envelope glycoprotein (JR-FL) at an equimolar concentration. Untreated controls were incubated with anti-gp41 Ab and goat anti-mouse Ab-coated Dynabeads or IgG-conjugated Dynabeads alone. At defined times (5−60 min) cells were centrifuged, and protein lysates were prepared and subjected to SDS-PAGE as previously described (23). Proteins were transferred to polyvinylidene difluoride membrane filters (Amersham Pharmacia Biotech, Piscataway, NJ), and membranes were blocked with 5% BSA in TBST before probing with phospho-FAK (pY397) Ab (Cell Signaling, Beverly, MA) or phospho-ZAP-70 Ab (pY397) Ab (BioSource International, Camarillo, CA) at room temperature for 1 h. After washing in TBST, membranes were incubated with HRP-conjugated goat anti-rabbit secondary Ab (Cell Signaling). After washing with TBS-T, signal detection was achieved with SuperSignal West Pico Chemiluminescent substrate (Pierce, Rockford, IL) and Biomax MR film (Eastman Kodak, Rochester, NY). The same blot was stripped with Restore Western blot stripping buffer (Pierce) for 30 min at room temperature and reblotted with anti-ZAP-70 Ab (Cell Signaling) or anti-FAK (A17; Santa Cruz Biotechnology, Santa Cruz, CA) for total ZAP-70 or FAK protein.

Results

Recombinant oligomeric rEnv from both X4 and R5 HIV strains induces infectious HIV from purified resting CD4+ T cells isolated from infected individuals

Oligomeric rEnv from the R5 HIV strains JR-FL and MW and from the T cell line-adapted X4 strain NL4.3 consistently induced p24 production from resting CD4+ T cells although JR-FL was most effective in the majority of experiments (Fig. 1A). Neither KLH (Fig. 1A), the CCR5 ligand chemokines (MIP-1α, MIP-1β, and RANTES; data not shown), nor the CXCR4 ligand stromal-derived factor-1 (data not shown) alone induced p24 production over levels observed in untreated cultures of resting CD4+ T cells. Oligomeric JR-FL rEnv induced a 3- to >100-fold increase (p = 0.04) in p24 production in cultures of resting CD4+ T cells isolated from 14 of 19 HIV-infected individuals (Fig. 1B). For comparison, PHA plus IL-2 stimulation of resting CD4+ T cells generally resulted in >1000-fold increase (p = 0.001) in p24 production in cultures of resting CD4+ T cells (Fig. 1B). No correlation was observed between the level of plasma viremia in a given donor and the level of p24 production induced by oligomeric rEnv treatment of the donors resting CD4+ T cells (data not shown).

Abbreviations used in this paper: MIP-1α, macrophage inflammatory protein-1α; iHIV, inactivated HIV; KLH, keyhole limpet hemocyanin; PI, propidium iodide.
To determine whether the p24 detected in the culture supernatant represented infectious virus, supernatants from KLH and oligomeric rEnv-stimulated resting CD4+ T cell cultures were filtered and placed onto anti-CD3 plus IL-2-stimulated HIV/H11002 T cell blasts. Passage of supernatants from oligomeric rEnv-treated, but not from KLH-treated, resting CD4+ T cell cultures isolated from 14 HIV-infected donors following exposure to oligomeric gp140 JR-FL (10 nM) or PHA plus IL-2. (Statistics were performed using Student’s t test.) C, Productive infection of HIV+ anti-CD3-stimulated PBMC following exposure to supernatant from gp140-stimulated, but not KLH-stimulated, resting CD4+ T cell (RT4) cultures. Data are representative of five independent experiments.

FIGURE 1. Oligomeric gp140 derived from both R5 and X4 HIV-1 strains induces p24 and infectious HIV production from resting CD4+ T cells isolated from HIV-infected donors. A, HIV-1 p24 production from resting CD4+ T cells isolated from four HIV-infected donors in untreated conditions or following exposure to gp140 protein (10 nM) derived from the R5 strains MW and JR-fb or the X4 strain NL4–3, a control protein, KLH (10 μg/ml), or mitogen (PHA plus IL-2). B, Levels of p24 produced by resting CD4+ T cells isolated from 14 HIV-infected donors following exposure to oligomeric gp140 JR-FL (10 nM) or PHA plus IL-2. (Statistics were performed using Student’s t test.) C, Productive infection of HIV+ anti-CD3-stimulated PBMC following exposure to supernatant from gp140-stimulated, but not KLH-stimulated, resting CD4+ T cell (RT4) cultures. Data are representative of five independent experiments.

To determine whether the p24 detected in the culture supernatant represented infectious virus, supernatants from KLH and oligomeric rEnv-stimulated resting CD4+ T cell cultures were filtered and placed onto anti-CD3 plus IL-2-stimulated HIV+ T cell blasts. Passage of supernatants from oligomeric rEnv-treated, but not from KLH-treated, resting CD4+ T cell cultures resulted in significant levels of viral replication (RT activity) in target HIV–PBMC cultures (Fig. 1C), demonstrating that a least a portion of the rEnv-induced virus was infectious.

Finally, to verify that oligomeric rEnv was inducing gene expression from provirus present in the resting CD4+ T cells rather than stimulating de novo infection by contaminating cell-bound virus, the reverse transcriptase inhibitor, 3TC, was added to the cultures of resting CD4+ T cells before exposure to BSA (protein control) or oligomeric rEnv. Culture supernatants were harvested after 3 days and assayed for viral RNA using a sensitive branched DNA technique (41). A 3- to 10-fold increase in supernatant-associated HIV RNA was observed 72 h following exposure of cells to oligomeric rEnv despite the presence of 3TC in the cultures (Fig. 2).

Oligomeric rEnv-mediated induction of HIV expression from HIV-infected resting CD4+ T cells is not associated with classical markers of T cell activation or proliferation

Resting CD4+ T cells were harvested at various time points following exposure to oligomeric rEnv or control mitogen and assessed for the expression of the cell surface activation markers CD25 (IL-2Rα-chain), CD69, and HLA-DR. In addition, culture supernatants were tested for the presence of IL-2 and IFN-γ cytokines typically produced by activated T cells; cellular division/proliferation was determined by [3H]thymidine uptake or by cell cycle analysis using flow cytometry. At no time following exposure to oligomeric rEnv did resting CD4+ T cells express the cell
surface activation Ags CD69, CD25, or HLA-DR (Fig. 3A). Furthermore, oligomeric rEnv-stimulated cells did not secrete detectable levels of IL-2 or IFN-γ (data not shown), nor did they undergo cellular division, as assessed by cell cycle analyses (flow cytometric analyses of PI incorporation; Fig. 3B) or [3H]thymidine uptake (data not shown). In this regard, >98.5% of both untreated and oligomeric rEnv-treated resting CD4\(^+\) T cells remained in G0/G1 stages of the cell cycle throughout the 10-day period of analysis.

Oligomeric rEnv-mediated induction of HIV expression from resting CD4\(^+\) T cells is not associated with apoptosis

To determine whether treatment of resting CD4\(^+\) T cells with oligomeric rEnv is associated with increased apoptosis, untreated or PHA-treated cells were exposed to envelope protein, and the PI-negative (nonnecrotic) population was analyzed for apoptosis by staining for annexin V, a marker of commitment to apoptosis. In agreement with previous studies (32), PHA-stimulated cells exhibited a high basal level of apoptosis that was further increased by exposure to HIV envelope (Fig. 4B). In contrast, resting CD4\(^+\) T cells in untreated conditions exhibited only low basal levels of apoptosis, and these levels were not increased following exposure to HIV envelope (Fig. 4A).

Detection of HIV oligomeric rEnv-mediated signal transduction

The ability of HIV gp140 to mediate cellular signal transduction, through both CD4 and chemokine receptors, has been investigated in numerous studies (reviewed in Ref. 28). However, to date the effects of HIV envelope on signal transduction in highly purified resting CD4\(^+\) T cells have not been described. Resting CD4\(^+\) T cells were exposed to 100 nM JR-FL HIV envelope plus anti-gp41-conjugated immunomagnetic beads or nonspecific mlgG-conjugated immunomagnetic beads for various periods of time, and cellular lysates were assessed for the presence of the phosphorylated forms of FAK and ZAP-70. As previously reported to occur in total CD4\(^+\) T cells (23), HIV JR-FL envelope induced the phosphorylation of both FAK and ZAP-70 resting CD4\(^+\) T cells (Fig. 5). These data demonstrate that certain of the oligomeric rEnv-mediated signal transduction events previously reported to occur in total CD4\(^+\) T cells (23, 26) are similarly induced in resting CD4\(^+\) T cells.

Discussion

The present study demonstrates that interaction of resting CD4\(^+\) T cells from HIV-infected individuals with HIV-1 oligomeric rEnv protein results in a level of cellular perturbation that is sufficient for the production of infectious HIV, but is below the threshold required to induce classic T cell activation and apoptosis. Such suboptimal cellular activation, triggered by envelope protein or other physiologic stimuli, may maintain low levels of viral replication from the resting CD4\(^+\) T cell HIV reservoir without altering the long-lived resting phenotype of this population.

In the present study oligomeric rEnv HIV envelope protein was found to stimulate virus production from resting CD4\(^+\) T cells isolated from HIV-infected donors in the absence of the induction of markers of classic T cell activation, such as the expression of CD25, CD69, or HLA-DR; progression out of G0/G1; or cytokine (IL-2, IFN-γ) secretion. The ability of in vivo-infected CD4\(^+\) T cells to produce infectious HIV in the absence of indicators of cellular activation has not been previously demonstrated. HIV production from resting CD4\(^+\) T cells isolated from HIV-positive donors can be induced following treatment of cells with mitogens (3–5) as well as with physiological agents such as cytokines (6, 43); however, in these studies virus expression was associated with the appearance of markers of classical T cell activation.

The effect of envelope protein (gp120 or gp120-anti-gp120 complexes) or inactivated HIV (iHIV) virions on HIV expression has been previously investigated using unstimulated PBMC isolated from HIV-infected donors (29) or PBMC infected with HIV in vitro (24). In the earlier study the expression of HIV was associated with a transition to the S/G2/M stage of the cell cycle and the expression of the cell surface activation marker CD25 (24). In contrast, HIV-uninfected PBMC treated with either iHIV or soluble gp120 failed to express the cell surface activation markers CD25 and CD69 (30), although a small increase in cellular proliferation was observed 48 h post-treatment with iHIV. The discrepancy between our data and those of the previous studies (24, 29, 30) with regard to the effects of envelope on cellular activation probably reflect differences in the cellular systems analyzed and, perhaps, the form in which envelope protein is presented. In PBMC an indirect effect on T cell activation may occur following the interaction between gp120/160 and other CD4\(^+\) cells, such as monocyte/macrophages. In this regard, treatment of monocyte/macrophages with gp41 (44) or with oligomeric rEnv (27) results in the up-regulation of T cell-activating factors such as the T cell costimulatory molecule, B7 (44), and the cytokine, IL-1β (27).

Recent studies using HIV-uninfected unstimulated PBMC have demonstrated that the cellular events triggered by interaction with oligomeric rEnv are much more extensive than previously thought and, overall, favor virus replication over cell death (27). HIV envelope-mediated alterations in function include changes in calcium mobilization (22, 25), activation of cytoskeleton components (23, 26, 27), induction of chemotaxis (22), and elevation of mRNA for a large number of kinases (27), RNA polymerase II (27), transcription elongation factors (27), long terminal repeat binding transcription factors (27), and HIV-inducing cytokines/chemokines and factors that control their secretion (27). Although not addressed directly in the present study, the central role of CD4 receptor-mediated signaling in HIV envelope-mediated induction of HIV reported by others (29) is supported by several observations in the present study: the ability of R5 HIV envelope to induce virus expression in resting CD4\(^+\) T cells that lack detectable CCR5 expression (data not shown) and the inability of CCR5 or CXCR4
ligand chemokines alone to activate HIV expression (data not shown). Furthermore, in the present study rEnv-mediated induction of HIV expression in resting CD4$^+$ T cells was not dependent on the activity of endogenous TNF-$\alpha$, as determined by neutralization experiments (data not shown); similar findings in studies using HIV-infected PBMC have been reported (39). The HIV envelope-mediated signal transduction events that are critical for HIV expression in purified quiescent T cells have yet to be elucidated.

While the triggering of certain cellular pathways by envelope protein may promote viral replication in relatively resting T cells, it may also result in abnormal immune function and/or apoptosis in T cells that are subsequently or were previously activated (Refs. 30–33; reviewed in Ref. 34). Of interest, iHIV virion-mediated CD4$^+$ T cell apoptosis in unstimulated PBMC was found to be primarily dependent on the presence of host MHC proteins incorporated on the surface of iHIV virions (30). Treatment of unstimulated CD4$^+$ T cells or PBMC with purified oligomeric rEnv triggers signaling pathways that both activate and suppress apoptosis (26, 27), suggesting that the balance of these two events may determine whether cell death is triggered. The data of the present study suggest that additional T cell signaling events are necessary for envelope-mediated induction of apoptosis in otherwise resting CD4$^+$ T cells. These observations are consistent with established literature linking cellular activation with increased sensitivity to programmed cell death (8). It remains to be determined whether interaction of resting CD4$^+$ T cells with HIV gp120/160 or virions in vivo reduces the relatively long half-life of this cellular population (3–5), at least with regard to apoptotic cell death.

The extent to which the interaction between CD4$^+$ T cells and HIV envelope promotes virus production in vivo requires further investigation. Oligomeric rEnv-mediated induction of HIV production does not require new infection of the target cell and could be mediated by defective virions or soluble or cell-bound protein, particularly in lymphoid tissue sites where HIV/protein remains trapped on follicular dendritic cells for extended periods of time (35). In addition, gp120/160 or gp120-anti-gp120 immune complexes can be detected in the serum (37), on the surface of CD4$^+$ T cells and monocytes (39), and in certain tissue sites (38) of HIV-infected individuals. Of particular interest is the observation that phenotypically resting CD4$^+$ T cells isolated from HIV-infected donors with detectable plasma viremia (>500 copies/ml) spontaneously secrete low levels of HIV virions (A. L. Kinter, unpublished observations). In this regard, resting CD4$^+$ T cells

**FIGURE 3.** The gp140-mediated induction of HIV expression in resting CD4$^+$ T cells is not associated with markers of cellular activation or progression out of the G0/G1 phase of the cell cycle. A, Five days following exposure to oligomeric gp140 (100 nM), resting CD4$^+$ T cells were assessed for the expression of CD4, HLA-DR, CD69, and CD25 by four-color flow cytometry; CD4$^+$ HLA-DR$^+$ expression (top plots) and coexpression of CD25 and CD69 (lower plots) are shown. Similar results were obtained at all time points tested; results are representative of nine independent experiments. B, Cell cycle analysis (PI incorporation) of untreated and gp140-treated (100 nM) resting CD4$^+$ T cells after 5 days in culture. Similar results were obtained at all time points tested; results are representative of five independent experiments.
isolated from a minority of viremic patients used in the present study expressed low levels of p24 in untreated and KLH-treated virus production ex vivo by resting CD4+ T cells of viremic individuals is due in part to cellular perturbation following interaction between resting CD4+ T cells and HIV virions or envelope with or without Ab complexes in vivo. The ability of HIV envelope to induce a state of cellular perturbation that is sufficient to support virus replication in HIV-infected resting CD4+ T cells without triggering apoptosis provides a mechanism for the virus itself to directly participate in the maintenance of HIV production from this cellular reservoir.

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