Short Communication

HIV Type 1 Accumulates in Influenza-Specific T Cells in Subjects Receiving Seasonal Vaccination in the Context of Effective Antiretroviral Therapy

R. Brad Jones,¹ Colin Kovacs,² Tae-Wook Chun,³ and Mario A. Ostrowski^{1,4}

Abstract

Whether or not HIV-1 continues to infect cells in individuals treated with effective antiretroviral therapy (ART) remains controversial. Here, we determined whether the redistribution of the HIV-1 proviral burden with respect to antigen specificity of CD4⁺ cells would provide evidence for ongoing infection cycles *in vivo*. HIV-1 preferentially infects antigen-stimulated CD4⁺ T cells. In the setting of prolonged effective ART, we postulated that if infection cycles were occurring, influenza-specific CD4⁺ T cells, activated by influenza vaccination, would preferentially accumulate proviral burden. Peripheral blood mononuclear cells (PBMCs) were collected from HIV-1-infected subjects who had been treated with effective ART for >5 years, before and after influenza vaccination. CD4⁺ T cells were sorted by antigen specificity and HIV-1 proviral burdens were determined. Levels of HIV-1 production upon *in vitro* antigenic stimulation were also measured. At baseline, influenza-specific CD4⁺ T cells carried higher HIV-1 proviral loads than HIV-1-p55-specific CD4⁺ T cells. Upon influenza vaccination we observed trends toward elevated levels of HIV-1 proviral DNA in influenza and HIV-1-p55-specific, but not tetanus toxoid or cytomegalovirus (CMV)-specific CD4⁺ T cells. Higher levels of HIV-1 virions were produced upon influenza stimulation in postvaccination as compared to baseline samples. While the trends toward increased proviral burdens in influenza-specific cells failed to reach statistical significance, our observation of disproportionately high levels of provirus in influenza-specific cells at baseline indicates that this may represent a real increase that is cumulative over multiple annual vaccinations. This has implications for the eradication of HIV-1 by adding to the evidence that the resting CD4⁺ T cell viral reservoir is continually replenished in ART-treated subjects.

Introduction

THE IMPLEMENTATION OF ANTIRETROVIRAL therapy (ART) routinely results in the suppression of plasma viremia to levels below the detection threshold of conventional assays, but is unable to eradicate HIV-1 infection. Long-lived resting CD4⁺ T cells in the periphery harbor a latent HIV-1 reservoir that may be responsible for reseeding systemic infection upon cessation of ART.^{1–3} Three lines of evidence indicate that low levels of HIV-1 replication occur even in the context of ART. First, unintegrated proviral DNA and cell-associated HIV-1 RNA have been identified in peripheral blood CD4⁺ T cells of infected individuals receiving ART, attesting to recent infection.^{1,4–10} Second, phylogenetic analyses have revealed reciprocal cross-infection between the

resting and activated CD4⁺ T cell compartment in ART-treated subjects.¹¹ Finally, a number of studies have demonstrated that the intensification of conventional ART in aviremic patients resulted in accelerated decay of HIV-1 in the latently infected CD4⁺ T cell compartment.^{12–14}

The existence of ongoing viral replication in ART-treated subjects does, however, remain an area of controversy with other studies showing no evidence for decay of residual plasma viremia (1–49 copies/ml) upon ART intensification.^{15–18} An argument has also been made against ongoing replication based on the observation of very low levels of influx of predominant plasma HIV-1 clones into the resting CD4⁺ T cell compartment.^{19–21} The resolution of this controversy is of practical importance in guiding treatment intensification, and has implications for the eradication of HIV-1 infection.

¹Department of Immunology, University of Toronto, Medical Sciences Building, Toronto, Ontario, Canada.

²Maple Leaf Medical Clinic, Toronto, Ontario, Canada.

³National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland.

⁴Li Ka Shing Knowledge Institute of St. Michael's Hospital, Toronto, Ontario, Canada.

TABLE 1. CLINICAL DATA FOR STUDY COHORT

Patient ID	Age (years)	Sex	Duration of ART (years)	Current ART regimen	Viral load (copies/ml)	Abs CD4 (cells/mm ³)	Samples taken
265	55	M	9	FTC/TDF/SQV/RTV	<50	540	Baseline only
73	43	M	10	FTC/ABC/SQV/LPV/RTV	<50	480	Baseline only
267	48	M	14	AZT/3TC/NFV	<50	400	Baseline only
215	53	M	17	FTC/ABC/TDF/LPV/RTV	<50	1220	Baseline only
275	63	M	9	ATV/r/3TC/AZT	<50	840	Baseline only
550	41	M	12	TDF/FTC/TMC114/RTV	<50	350	Baseline and postvaccination
549	40	M	22	ABC/3TC/LPV/RTV	<50	1390	Baseline and postvaccination
238	47	M	9	ABC/3TC/AZT	<50	820	Baseline and postvaccination
288	47	M	9	AZT/3TC/LPV/RTV	<50	700	Baseline and postvaccination
298	39	M	5	TDF/FTC/ATV/RTV	<50	830	Baseline and postvaccination
292	49	M	13	ABC/3TC/ATZ/RTV/EFZ	<50	590	Baseline and postvaccination

ART, antiretroviral therapy.

We tested the hypothesis that ongoing HIV-1 replication in the context of prolonged effective ART would result in the redistribution of proviral load among CD4⁺ T cells with respect to antigen specificities. In acute and chronic untreated HIV-1 infection, as well as in individuals treated with ART for a relatively short duration, it has been demonstrated that HIV-1-specific CD4⁺ T cells carry disproportionately high proviral burdens as compared to total memory CD4⁺ T cells and cytomegalovirus (CMV)-specific CD4⁺ T cells.²² This is thought to be largely due to the antigen-driven activated state of HIV-1-specific cells in the context of ongoing replication, given that activated CD4⁺ T cells are much more susceptible to infection than their resting counterparts.^{23–27} Although the majority of these infected cells die, a subset becomes quiescent and contributes to the resting memory CD4⁺ T cell latent reservoir. We reasoned that the paucity of HIV-1 antigens in subjects treated with effective ART would result in a substantial reduction in this bias, and hence that ongoing low-level replication would be manifested as a more even distribution of proviral burden between HIV-1-specific CD4⁺ T cells and cells of other specificities. We further posited that if low-level HIV-1 replication was occurring in ARV-treated subjects, CD4⁺ T cells specific for antigens that were frequently encountered in this context would continue to accumulate proviral load. We were able to test this for influenza-specific CD4⁺ T cells *in vivo* by taking advantage of the seasonal vaccination received by our cohort of ARV-treated subjects. Notably, several groups have demonstrated that vaccination with recall antigens, including either influenza or tetanus toxoid, results in the induction of HIV-1 replication in the absence of ART as evidenced by increases in both plasma viremia and proviral loads.^{28–34}

Materials and Methods

Subjects and samples

This study was approved by the University of Toronto Institutional Review Board. Subjects were recruited based on the criteria of (1) treated with ART (viral load <50 copies/mm³) for >5 years and (2) received annual influenza vaccinations for >5 years. Clinical data are presented in Table 1. Leukapheresis was performed on subjects prior to and 2 weeks after receiving a standard dose of Fluviral influenza vaccine, a trivalent inactivated vaccine (Shire Biologics).

Assessing levels of virus production upon antigenic stimulation

Peripheral blood mononuclear cells (PBMCs) were cultured at 5×10^6 cells/ml in stimulation medium [RPMI + 10% human AB serum + 1 μ g/ml anti-CD28 + 1 μ g/ml anti-CD49d + 1 μ M zidovudine (AZT) + 1 μ M lamivudine (3TC) + 1 μ M nevirapine (NVP)] with either 1 μ g/ml cytochrome *c* (Austral Biologicals), 1 μ g/ml HIV-1-p55 (Austral Biologicals), 1 μ g/ml tetanus toxoid (List Biological Laboratories), 1/50 dilution of Fluviral influenza vaccine (Shire Biologics), or 0.2 μ g/ml staphylococcal enterotoxin B (SEB) (Sigma). Supernatants were collected following 7 days of stimulation and viral RNA was quantified using the AMPLICOR-HIV Monitor test (Roche).

Assessing levels of provirus in antigen-specific cells

CD8⁺ T cells were depleted from PBMCs using Dynal beads (Invitrogen). The remaining cells were plated at 1×10^7 cells/ml in stimulation medium with either: cytochrome *c*, HIV-1-p55, tetanus toxoid, or Fluviral influenza vaccine at the concentrations indicated above, or with a 1/20 dilution of CMV antigen (Virion). These were enriched for antigen-specific cells using a cocktail of the interferon (IFN)- γ , tumor necrosis factor (TNF)- α , and interleukin (IL)-2 cytokine secretion assay enrichment and detection kits (Miltenyi Biotec). We achieved the following purities of phycoerythrin (PE)-labeled cells following enrichment (mean, range): HIV-1-p55—75.8%, 51.2–91.1%, influenza—94.5%, 90.8–96.6%, CMV—71.6%, 43.3–92.3%, and tetanus toxoid—55.3%, 48.6–78.4%. Selected populations were lysed and treated with proteinase K (Roche Applied Science) for 1 h at 56°C followed by heat inactivation. Proviral DNA was quantified by real-time polymerase chain reaction (PCR), as previously described.³⁵

Statistical analysis

Statistical tests were performed with GraphPad Prism software version 4.0. The tests used for each analysis are indicated in the legend of Fig. 1.

Results

PBMCs were obtained from 11 HIV-1-infected subjects who had (1) been on effective ART for >5 years and (2) received

FIG. 1. Quantitation of HIV-1 proviral loads and levels of virus production by antigen-specific CD4⁺ T cells at baseline and 2 weeks postinfluenza vaccination. **(A)** CD4⁺ T cells that produced interferon (IFN)- γ , tumor necrosis factor (TNF)- α , or interleukin (IL)-2 in response to antigen were labeled with phycoerythrin (PE) using a cytokine capture detection kit (Miltenyi Biotec) and then enriched using anti-PE magnetic beads. Shown are flow cytometry data from a representative experiment, sorting for HIV-1-p55-specific CD4⁺ T cells displaying unselected, negatively selected, and positively selected populations. The depicted populations have been gated on CD3⁺ CD4⁺ viable (7AAD-neg) lymphocytes. **(B)** Baseline (prevaccination) levels of HIV-1 proviral DNA in sorted HIV-1-p55, tetanus toxoid, and influenza specific CD4⁺ T cells were determined by qPCR. In a subset of subjects, proviral loads were also determined in unfractionated CD4⁺ T cells. p values were calculated by Wilcoxon matched pairs signed-rank test. **(C)** Levels of HIV-1 proviral DNA in sorted antigen-specific CD4⁺ T cells were determined by qPCR at baseline (before) and 2 weeks postinfluenza vaccination (after). p values were calculated by paired t -test (one-tailed). **(D)** Effect of influenza vaccination on virus production upon antigenic stimulation. Samples were taken from long-term antiretroviral therapy (ART)-suppressed subjects before and 2 weeks after influenza vaccination. CD8-depleted peripheral blood mononuclear cells (PBMCs) were stimulated for 7 days in the presence of antiretroviral (ARV) drugs. Supernatants were collected and viral RNA was quantified by the AMPLICOR-HIV Monitor test (Roche). p values were calculated by paired t -test (one-tailed).

annual influenza vaccinations for >5 years. For four of these subjects, samples were collected immediately before and 2 weeks postinfluenza vaccination. Levels of baseline HIV-1 proviral DNA were quantified in sorted HIV-1-p55, tetanus toxoid, and influenza-specific CD4⁺ T cells. We observed that

HIV-1-p55-specific cells carried the lowest proviral burden with means of HIV-1-p55—564.5 copies/10⁶ CD4⁺ T cells, tetanus toxoid—1,081.8 copies/10⁶ CD4⁺ T cells, and influenza—1,685.2 copies/10⁶ CD4⁺ T cells. The differences between proviral loads in HIV-1-p55 versus influenza specific

cells were significant ($p=0.013$), while the differences between HIV-1-p55 and tetanus toxoid were not ($p=0.062$) (Fig. 1A and B). Thus, in subjects treated with effective ART for prolonged periods of time, influenza-specific CD4⁺ T cells carry a higher proviral burden than HIV-1-p55-specific CD4⁺ T cells. Two weeks postinfluenza vaccination we observed trends toward increased proviral loads in HIV-1-p55-specific (mean of 419.3 copies/10⁶ CD4⁺ T cells prevaccination versus 2,108.1 postvaccination, $p=0.135$, increases observed in four/four subjects, Fig. 1C) and influenza-specific CD4⁺ T cells (mean of 1,675.8 copies/10⁶ CD4⁺ T cells prevaccination versus 3,125.5 postvaccination, increases observed in three/four subjects, $p=0.135$, Fig. 1C). Neither tetanus toxoid-specific, CMV-specific, nor SEB-responsive CD4⁺ T cells displayed a consistent trend (tetanus toxoid—mean of 328.1 copies/10⁶ CD4⁺ T cells prevaccination versus 383.5 postvaccination, $p=0.305$, CMV—mean of 2,113.2 copies/10⁶ CD4⁺ T cells prevaccination versus 2,215.8 postvaccination, $p=0.417$, SEB—mean of 1,839.8 copies/10⁶ CD4⁺ T cells prevaccination versus 1,775.5 postvaccination, $p=0.403$, Fig. 1C). Therefore, although we detected trends toward increased HIV-1 proviral burden in influenza and HIV-1-p55-specific CD4⁺ T cells, these were not significant in our small sample size.

An increase in proviral burden in antigen-specific CD4⁺ T cells upon vaccination would also manifest as increased viral production upon *in vitro* antigenic stimulation. This has previously been reported for vaccination with tetanus toxoid.³¹ To test this, CD8-depleted PBMCs were stimulated for 7 days in the presence of 3TC, AZT, and nevirapine and levels of HIV-1 RNA in supernatants were quantified. We observed consistently higher levels of virus production upon influenza stimulation in postvaccination versus prevaccination samples (means of 125 versus 35 copies/ml, $p=0.003$, Fig. 1D). We did not observe a consistent or significant increase in levels of virus production following influenza vaccination upon stimulation with tetanus toxoid, CMV, or SEB (Fig. 1D).

Stimulation with HIV-1-p55 produced a striking result, where postinfluenza vaccination samples from two subjects produced very high levels of HIV-1 RNA in the range of millions of copies/ml. Two other subjects exhibited modest increases in virus production upon HIV-1-p55 stimulation postinfluenza vaccination and one exhibited a decrease. Thus, influenza vaccination enhanced virus production from both HIV-1 and influenza-specific cells, after restimulation with their respective antigens. This effect was antigen specific as we did not observe increased levels of virus production postvaccination upon *in vitro* stimulation with tetanus toxoid or CMV.

Discussion

We observed that in subjects treated with ART for prolonged periods of time (>5 years), influenza-specific CD4⁺ T cells carried a substantially higher proviral burden than their HIV-1-p55-specific counterparts. This is in contrast to the disproportionately high levels of proviral DNA in HIV-1-specific CD4⁺ T cells reported in untreated HIV-1 infection and in subjects treated with ART for relatively short durations.²² This supports the idea that the recurrent stimulation of the influenza-specific CD4⁺ T cells population, through annual vaccination and influenza infection, renders them susceptible to infection by low levels of ongoing HIV-1 rep-

lication, resulting in the accumulation of proviral DNA. We tested this *in vivo* by determining whether we could observe an increase in HIV-1 proviral load in influenza-specific CD4⁺ T cells in ARV-treated subjects following a single influenza vaccination. Although we did observe a trend toward an increased level of provirus in influenza-specific cells, which was not observed in tetanus toxoid or CMV-specific cells, this did not reach statistical significance. This may reflect the inadequate statistical power of our sample size to detect a relatively small *in vivo* increase in proviral levels over a single round of vaccination. This is supported by our observation of significantly higher levels of proviral DNA in influenza-specific cells in baseline than cells of other specificities, consistent with a cumulative effect over multiple years of annual vaccination.

We observed significantly higher levels of HIV-1 production upon *in vitro* influenza stimulation in postvaccination versus prevaccination samples. Since these stimulations were performed in the presence of 3TC, AZT, and nevirapine it is unlikely that *de novo* infection of vaccine-primed influenza-specific cells occurred *in vitro*. We propose that this likely reflects *in vivo* infection of vaccine-primed influenza-specific CD4⁺ T cells, and thus a larger pool of infected influenza-specific CD4⁺ T cells at initiation of the *in vitro* culture. This is supported by the trends toward higher levels of provirus in influenza-specific CD4⁺ T cells observed following vaccination. As the production of HIV-1 over 7 days comprises an amplification step, this may have improved our ability to detect a relatively small *in vivo* increase in infected influenza-specific CD4⁺ T cells following a single vaccination. We cannot rule out, however, an indirect effect whereby higher levels of cytokine production postvaccination may drive the production of HIV-1 from non-influenza-specific cells present in the culture.

It is also of interest to consider the possibility of a secondary effect of influenza vaccination, whereby the induction of HIV-1 replication by influenza vaccination activates HIV-1-specific CD4⁺ T cells and recruits them to sites of replication, rendering these more susceptible to infection. We observed a trend toward increased levels of HIV-1 proviral DNA in HIV-1-p55-specific CD4⁺ T cells upon influenza vaccination. This does not appear to agree, however, with our data showing relatively low levels of provirus in HIV-1-p55-specific CD4⁺ T cells at baseline. It is conceivable that HIV-1-p55-specific CD4⁺ T cells become infected upon influenza vaccination, due to such a secondary effect, but are less prone to returning to a resting memory state. In this regard, it is interesting to note that two subjects exhibited extraordinarily high levels of HIV-1 production upon HIV-1-p55 stimulation of postvaccination samples. We suggest that cells producing such high levels of HIV-1 are unlikely to survive and return to a resting state. The reason for this high level of HIV-1 production from HIV-1-p55-specific cells is presently unclear and will be the subject of future studies.

Our data add to the evidence that low-levels of HIV-1 replication occur in ARV-treated subjects following antigenic stimulation, comprising a barrier to eradication. Our findings should not deter current recommendations for routine vaccination of HIV-1-infected individuals for prevention of influenza or pneumococcal pneumonia.³⁶ We and others have previously demonstrated that although vaccinations can transiently enhance HIV-1 viral replication due to vaccine-induced immune activation, the immune protection conferred

by vaccine-induced antibodies will prevent the more prolonged immune the activation and enhanced viral replication that can result from the full blown infections that the vaccines were meant to prevent.^{30,31,33,37} In this article we show that although vaccination can prevent the deleterious effects of acute bacterial and viral infections, HIV-1 can still exploit newly primed antigen-specific memory cells resulting from vaccination for its own use, by establishing new reservoirs. However, the overall effect of vaccination may still be to limit HIV-1 viral reservoirs in the long run, at the expense of infecting a small population of vaccine-specific cells.

Acknowledgments

Funding for this work was obtained from the Canadian Institutes of Health Research. The following reagents were obtained from the NIH AIDS Research and Reference Reagent Program, Division of AIDS, NIAID, NIH: nevirapine and 3TC. R.B.J. gratefully acknowledges salary support from the Ontario HIV Treatment Network (OHTN). Biosafety level 3 laboratory space was provided by the Canadian Foundation for HIV Research (CANFAR) in partnership with the Ontario Innovation Trust and the Canadian Foundation for Innovation.

Author Disclosure Statement

No competing financial interests exist.

References

- Chun TW, Stuyver L, Mizell SB, Ehler LA, Mican JA, Baseler M, *et al.*: Presence of an inducible HIV-1 latent reservoir during highly active antiretroviral therapy. *Proc Natl Acad Sci USA* 1997;94:13193–13197.
- Finzi D, Hermankova M, Pierson T, Carruth LM, Buck C, Chaisson RE, *et al.*: Identification of a reservoir for HIV-1 in patients on highly active antiretroviral therapy. *Science* 1997;278:1295–1300.
- Wong JK, Hezareh M, Gunthard HF, Havlir DV, Ignacio CC, Spina CA, and Richman DD: Recovery of replication-competent HIV despite prolonged suppression of plasma viremia. *Science* 1997;278:1291–1295.
- Buzon MJ, Massanella M, Llibre JM, Esteve A, Dahl V, Puertas MC, *et al.*: HIV-1 replication and immune dynamics are affected by raltegravir intensification of HAART-suppressed subjects. *Nat Med* 2010;16:460–465.
- Furtado MR, Callaway DS, Phair JP, Kunstman KJ, Stanton JL, Macken CA, *et al.*: Persistence of HIV-1 transcription in peripheral-blood mononuclear cells in patients receiving potent antiretroviral therapy. *N Engl J Med* 1999;340:1614–1622.
- Natarajan V, Bosche M, Metcalf JA, Ward DJ, Lane HC, and Kovacs JA: HIV-1 replication in patients with undetectable plasma virus receiving HAART. Highly active antiretroviral therapy. *Lancet* 1999;353:119–120.
- Patterson BK, McCallister S, Schutz M, Siegel JN, Shults K, Flener Z, and Landay A: Persistence of intracellular HIV-1 mRNA correlates with HIV-1-specific immune responses in infected subjects on stable HAART. *AIDS* 2001;15:1635–1641.
- Sharkey M, Triques K, Kuritzkes DR, and Stevenson M: In vivo evidence for instability of episomal human immunodeficiency virus type 1 cDNA. *J Virol* 2005;79:5203–5210.
- Sharkey ME, Teo I, Greenough T, Sharova N, Luzuriaga K, Sullivan JL, *et al.*: Persistence of episomal HIV-1 infection intermediates in patients on highly active anti-retroviral therapy. *Nat Med* 2000;6:76–81.
- Zhang L, Ramratnam B, Tenner-Racz K, He Y, Vesanen M, Lewin S, *et al.*: Quantifying residual HIV-1 replication in patients receiving combination antiretroviral therapy. *N Engl J Med* 1999;340:1605–1613.
- Chun TW, Nickle DC, Justement JS, Large D, Semerjian A, Curlin ME, *et al.*: HIV-infected individuals receiving effective antiviral therapy for extended periods of time continually replenish their viral reservoir. *J Clin Invest* 2005;115:3250–3255.
- Yukl SA, Gianella S, Sinclair E, Epling L, Li Q, Duan L, *et al.*: Differences in HIV burden and immune activation within the gut of HIV-positive patients receiving suppressive antiretroviral therapy. *J Infect Dis* 2010;202:1553–1561.
- Havlir DV, Strain MC, Clerici M, Ignacio C, Trabattini D, Ferrante P, and Wong JK: Productive infection maintains a dynamic steady state of residual viremia in human immunodeficiency virus type 1-infected persons treated with suppressive antiretroviral therapy for five years. *J Virol* 2003;77:11212–11219.
- Ramratnam B, Ribeiro R, He T, Chung C, Simon V, Vanderhoeven J, *et al.*: Intensification of antiretroviral therapy accelerates the decay of the HIV-1 latent reservoir and decreases, but does not eliminate, ongoing virus replication. *J Acquir Immune Defic Syndr* 2004;35:33–37.
- Dinso JB, Kim SY, Wiegand AM, Palmer SE, Gange SJ, Cranmer L, *et al.*: Treatment intensification does not reduce residual HIV-1 viremia in patients on highly active antiretroviral therapy. *Proc Natl Acad Sci USA* 2009;106:9403–9408.
- Hatano H, Hayes TL, Dahl V, Sinclair E, Lee TH, Hoh R, *et al.*: A randomized, controlled trial of raltegravir intensification in antiretroviral-treated, HIV-infected patients with a suboptimal CD4+ T cell response. *J Infect Dis* 2011;203:960–968.
- McMahon D, Jones J, Wiegand A, Gange SJ, Kearney M, Palmer S, *et al.*: Short-course raltegravir intensification does not reduce persistent low-level viremia in patients with HIV-1 suppression during receipt of combination antiretroviral therapy. *Clin Infect Dis* 2010;50:912–919.
- Gandhi RT, Bosch RJ, Aga E, Albrecht M, Demeter LM, Dykes C, *et al.*: No evidence for decay of the latent reservoir in HIV-1-infected patients receiving intensive enfuvirtide-containing antiretroviral therapy. *J Infect Dis* 2009;201:293–296.
- Bailey JR, Sedaghat AR, Kieffer T, Brennan T, Lee PK, Wind-Rotolo M, *et al.*: Residual human immunodeficiency virus type 1 viremia in some patients on antiretroviral therapy is dominated by a small number of invariant clones rarely found in circulating CD4+ T cells. *J Virol* 2006;80:6441–6457.
- Joos B, Fischer M, Kuster H, Pillai SK, Wong JK, Boni J, *et al.*: HIV rebounds from latently infected cells, rather than from continuing low-level replication. *Proc Natl Acad Sci USA* 2008;105:16725–16730.
- Sedaghat AR, Siliciano JD, Brennan TP, Wilke CO, and Siliciano RF: Limits on replenishment of the resting CD4+ T cell reservoir for HIV in patients on HAART. *PLoS Pathog* 2007;3:e122.
- Douek DC, Brenchley JM, Betts MR, Ambrozak DR, Hill BJ, Okamoto Y, *et al.*: HIV preferentially infects HIV-specific CD4+ T cells. *Nature* 2002;417:95–98.

23. Claydon EJ, Bennett J, Gor D, and Forster SM: Transient elevation of serum HIV antigen levels associated with intercurrent infection. *AIDS* 1991;5:113–114.
24. Fultz PN, Gluckman JC, Muchmore E, and Girard M: Transient increases in numbers of infectious cells in an HIV-infected chimpanzee following immune stimulation. *AIDS Res Hum Retroviruses* 1992;8:313–317.
25. Stevenson M, Stanwick TL, Dempsey MP, and Lamonica CA: HIV-1 replication is controlled at the level of T cell activation and proviral integration. *EMBO J* 1990;9:1551–1560.
26. Zack JA, Arrigo SJ, Weitsman SR, Go AS, Haislip A, and Chen IS: HIV-1 entry into quiescent primary lymphocytes: Molecular analysis reveals a labile, latent viral structure. *Cell* 1990;61:213–222.
27. Zagury D, Bernard J, Leonard R, Cheynier R, Feldman M, Sarin PS, and Gallo RC: Long-term cultures of HTLV-III-infected T cells: A model of cytopathology of T-cell depletion in AIDS. *Science* 1986;231:850–853.
28. Ho DD: HIV-1 viraemia and influenza. *Lancet* 1992;339:1549.
29. O'Brien WA, Grovit-Ferbas K, Namazi A, Ovcak-Derzic S, Wang HJ, Park J, *et al.*: Human immunodeficiency virus-type 1 replication can be increased in peripheral blood of seropositive patients after influenza vaccination. *Blood* 1995;86:1082–1089.
30. Ostrowski MA, Krakauer DC, Li Y, Justement SJ, Learn G, Ehler LA, *et al.*: Effect of immune activation on the dynamics of human immunodeficiency virus replication and on the distribution of viral quasiespecies. *J Virol* 1998;72:7772–7784.
31. Ostrowski MA, Stanley SK, Justement JS, Gantt K, Goletti D, and Fauci AS: Increased in vitro tetanus-induced production of HIV type 1 following in vivo immunization of HIV type 1-infected individuals with tetanus toxoid. *AIDS Res Hum Retroviruses* 1997;13:473–480.
32. Rosok B, Voltersvik P, Bjerknes R, Axelsson M, Haaheim LR, and Asjo B: Dynamics of HIV-1 replication following influenza vaccination of HIV+ individuals. *Clin Exp Immunol* 1996;104:203–207.
33. Stanley SK, Ostrowski MA, Justement JS, Gantt K, Hedayati S, Mannix M, *et al.*: Effect of immunization with a common recall antigen on viral expression in patients infected with human immunodeficiency virus type 1. *N Engl J Med* 1996;334:1222–1230.
34. Staprans SI, Hamilton BL, Follansbee SE, Elbeik T, Barbosa P, Grant RM, and Feinberg MB: Activation of virus replication after vaccination of HIV-1-infected individuals. *J Exp Med* 1995;182:1727–1737.
35. Chun TW, Justement JS, Murray D, Hallahan CW, Maenza J, Collier AC, *et al.*: Rebound of plasma viremia following cessation of antiretroviral therapy despite profoundly low levels of HIV reservoir: implications for eradication. *AIDS* 2010;24:2803–2808.
36. Kaplan JE, Benson C, Holmes KH, Brooks JT, Pau A, and Masur H: Guidelines for prevention and treatment of opportunistic infections in HIV-infected adults and adolescents: Recommendations from CDC, the National Institutes of Health, and the HIV Medicine Association of the Infectious Diseases Society of America. *MMWR Recomm Rep* 2009;58:1–207; quiz CE201–204.
37. Brichacek B, Swindells S, Janoff EN, Pirruccello S, and Stevenson M: Increased plasma human immunodeficiency virus type 1 burden following antigenic challenge with pneumococcal vaccine. *J Infect Dis* 1996;174:1191–1199.

Address correspondence to:

R. Brad Jones

Department of Immunology

University of Toronto

Medical Sciences Building, Room 6271

1 King's College Circle

Toronto, Ontario M5S 1A8

Canada

E-mail: brad.jones@utoronto.ca