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Tetanus vaccination with IL-2 during highly active antiretroviral therapy induces sustained and pronounced specific CD4 T-cell responses

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HIV-1 infection results in profound dysfunction of the CD4 T-cell population. Although highly active antiretroviral therapy allows the reconstitution of CD4 T-cell numbers, functional abnormalities remain, including inadequate responses to vaccination. IL-2 increases CD4 T-cell numbers and function. We report the effects of IL-2 given with tetanus vaccination in two patients in a larger immunotherapy study. In one case IL-2 induced sustained responses, which may have been caused by the timing of IL-2 with vaccination.

CD4 lymphocyte proliferative responses (LPR) to various stimuli are lost during HIV-1 infection, as immunodeficiency and the risk of opportunistic disease increases [1–5]. Reductions in the incidence of opportunistic disease result from highly active antiretroviral therapy (HAART), concomitant with the suppression of viral load and the normalization of CD4 cell counts [6,7]. Subsequently LPR are partly restored in many individuals [8–11].

We previously demonstrated that within the first 4 months of HAART renewed recall responses were specific to long-term antigens, such as herpes simplex virus and *Candida* [11]. Responses to short-lived antigens, such as purified protein derivative (PPD), tetanus or influenza, remained weak. In a different study [12], vaccination with short-term/neoantigens including tetanus after HAART was only able to yield poor LPR.

IL-2 therapy with HAART increased CD4 T-cell numbers together with mitogen and recall LPR [13–19]. We report here the data from two recipients of tetanus vaccination during a larger immunotherapy study. In one case, HAART resulted in a large reconstitution of LPR. Subsequent tetanus vaccination followed by IL-2 administration dramatically preserved tetanus-specific LPR, consistent with previous findings suggesting that the timing of IL-2 administration with the T-cell expansion/contraction phase of an immune response may be important [20]. For both tetanus vaccinated patients, we also show results from two IL-

2-treated, non-tetanus vaccinated, age and baseline CD4 cell count matched controls. Patients initiated HAART (at least one protease inhibitor or non-nucleoside reverse transcriptase inhibitor and two nucleoside analogues) at day 0. Blood samples were obtained at regular timepoints for viral load measurement using the Quantiplex HIV RNA 3.0 Chiron branched DNA assay (Chiron Diagnostics, Halstead, UK), lymphocyte subset quantification (Tetra One, Beckman Coulter, High Wycombe, UK), and lymphocyte proliferation assays as previously described [21], using tetanus (Aventis Pasteur, Marcy l'Etoile, France), PPD (Sigma, Poole, UK) and influenza A (Central Public Health Laboratory Service, London, UK) antigens. IL-2 was administered to patients subcutaneously at 5×10^6 units, twice a day, for 5 days, in three cycles, 4-weekly.

At baseline for HAART, patient 1 had a CD4 cell count of 358 cells/ μ l of whole blood and a viral load of 76 430 copies/ml plasma. Tetanus vaccination was administered at day 84 (444 CD4 cells/ μ l, viral load 253 copies/ml). IL-2 was subsequently administered at days 120 (742 CD4 cells, viral load 100 copies/ml), 161 and 189, increasing the CD4 cell count to 1465 cells/ μ l at day 219. Patient 2 initiated HAART with a significantly lower baseline CD4 cell count of 83 cells/ μ l, and a viral load of 100 801 copies/ml. The viral load became sustained below the limits of detection 126 days after the initiation of HAART. IL-2 was administered at days 196 (258 CD4 cells/ μ l), 252 and 280. Tetanus vaccination was subsequently administered at day 303 (543 CD4 cells/ μ l, remaining stable thereafter). Patients 3 and 4 were the controls for patient 1. Patient 3 (baseline CD4 cell count 279 cells/ μ l, viral load 76 430 copies/ml) received IL-2 at days 118 (456 CD4 cells/ μ l, viral load sustained at < 50 copies/ml), 146 and 174. The CD4 cell count subsequently rose to 862 cells/ μ l at day 202. Patient 4 (baseline CD4 cell count 412 cells/ μ l, viral load 122 459 copies/ml) received IL-2 at days 137 (456 CD4 cells/ μ l, viral load < 50), 172 and 201. The CD4 cell count rose to 1627 cells/ μ l at day 315. Patients 5 and 6 were the controls for patient 2. Patient 5 (baseline CD4 cell count 129 cells/ μ l, viral load 264 579 copies/ml) received IL-2 at days 110 (352 CD4 cells/ μ l, viral load < 50 copies/ml), 144 and 181. Patient 6 (baseline CD4 cell count 168 cells/ μ l, viral load 330 754 copies/ml) received IL-2 at days 167 (CD4 cell count 278, viral load < 50 copies/ml, sustained thereafter), 196 and 224. As with

patient 2, any IL-2-induced CD4 cell elevations were limited.

LPR results are presented as the stimulation index, calculated as the experimental counts per minute (cpm)/background cpm. A positive LPR equals a stimulation index of 5 or greater. Considerable increases in recall LPR to PPD, influenza and tetanus antigens were seen after the initiation of HAART for patient 1 (Fig. 1a). All these responses diminished after 112 days of HAART, despite tetanus vaccination at day 84. PPD and influenza responses remained weak throughout the remainder of the study. In stark contrast, the tetanus response increased substantially with each cycle of IL-2, and remained elevated until 350 days, before eventually declining.

The controls for patient 1, patients 3 and 4, typically

demonstrated similar transient 'bursts' in LPR shortly after the initiation of HAART (Fig. 1b and c), as previously described by us [11]. This burst was considerably lower for patient 4, and for both patients diminished after approximately 2 weeks. The tetanus response was similar to the 'flu and PPD responses.

The recall responses of patient 2 (Fig. 1d) were minimal in comparison with patients 1, 3 and 4. There was no improvement after HAART despite tetanus vaccination and IL-2 therapy. This patient's controls, patients 5 and 6, demonstrated a similar pattern.

The ability of patients 1 and 2 to respond to tetanus vaccination was notably different despite full viral suppression and good CD4 cell increases in response to IL-2. Patient 1 demonstrated a robust tetanus response, which, although subsequently waning, was substantially

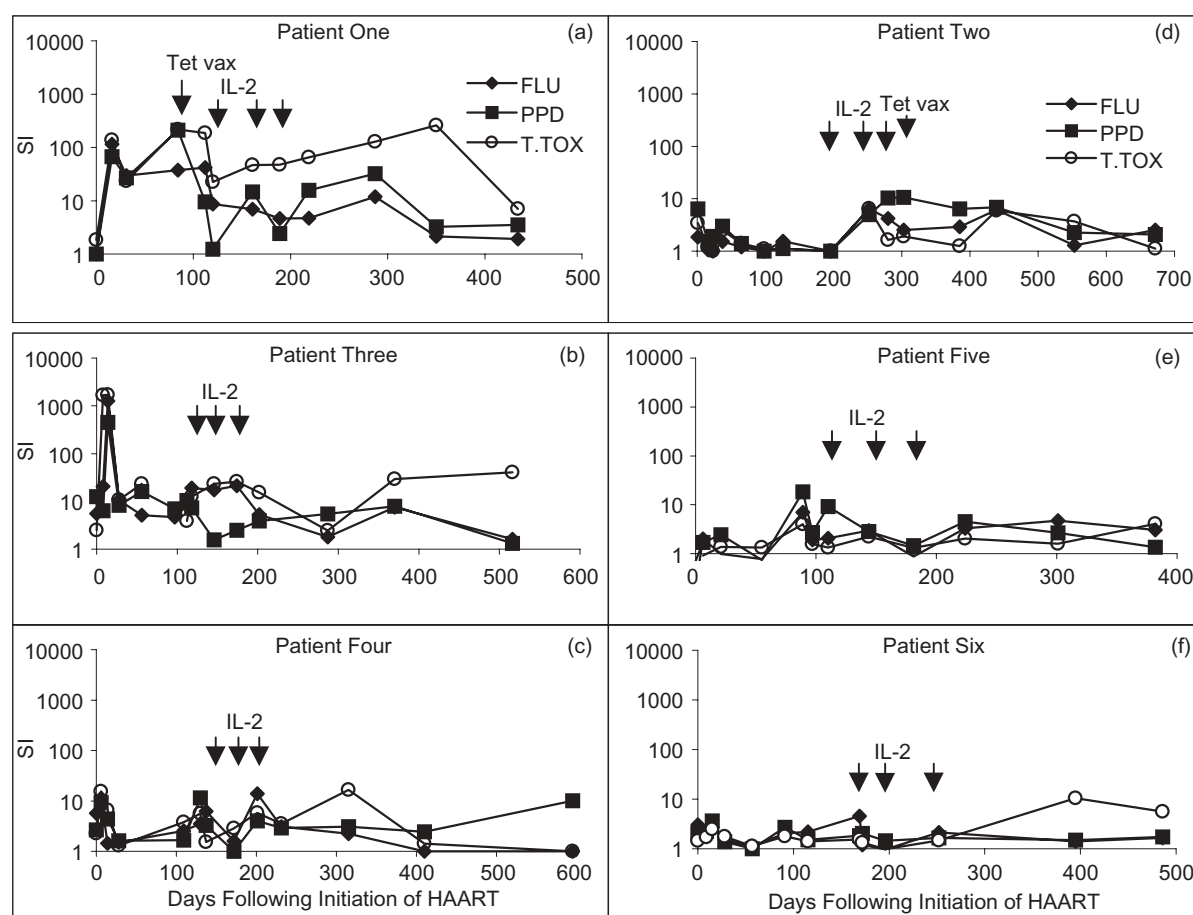


Fig. 1. Lymphocyte proliferative responses to tetanus (○), influenza (◆) and purified protein derivative antigens (■) are shown for patient 1 (a), controls for patient 1, patients 3 (b) and 4 (c), patient 2 (d) and controls for patient 2, patients 5 (e) and 6 (f). Tetanus vaccination was administered to patient 1 at day 84, and to patient 2 at day 303 as indicated by arrows. Five day courses of 5×10^6 twice a day subcutaneous IL-2 were administered to patient 1 at days 120, 161 and 189 and to patient 2 at days 196, 252 and 280 as indicated by arrows. Arrows similarly indicate the time of beginning of each IL-2 cycle for the two sets of controls. HAART, Highly active antiretroviral therapy; SI, stimulation index.

enhanced by the later administration of IL-2 for a further 250 days. Patient 2 demonstrated a negligible response to tetanus vaccination. Indeed 'flu and PPD responses also failed to recover despite the administration of IL-2.

Patient 2's low baseline CD4 cell count was a likely factor. Recall LPR are significantly effected by pre-HAART CD4 nadirs of less than 300 cells/ μ l [3,22]. Patients 3 and 4, with comparable baseline CD4 cell counts to patient 1, demonstrated moderate recall responses. Patients 5 and 6, with comparable low baseline CD4 cell counts to patient 2, demonstrated minimal recall responses after HAART and IL-2 therapy.

It is possible that these discordant vaccination responses may be partly affected by the timing of IL-2 with vaccination. Some studies have demonstrated that IL-2 administered before vaccination in HAART-treated HIV-1 patients does not increase LPR [17,18]. We propose that the administration of intermediate-dose IL-2 after vaccination may be more favourable. This is in line with the thinking that exogenous IL-2 administration during antigen-specific T-cell contraction significantly lengthens responses [20]. Patient 1's tetanus response increased as predicted by this model.

Therefore, in those with higher CD4 cell nadirs before HAART, vaccine responses that are insufficient may be modifiable. In addition, because sustained tetanus-specific responses are inducible in HIV-1 infection, tetanus vaccination may serve as a useful control in therapeutic HIV-1 vaccine trials. Although larger studies are required to validate this case report, we suggest that IL-2 administered after vaccination in patients with higher CD4 cell nadirs may be most effective.

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HIV viral shedding in semen: lack of correlation with systemic virus-specific CD8 responses

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Semen is a major transmission vector for HIV. Virus-specific CD8 T cells are critical in HIV control, but their relationship with semen viral load is unknown. We therefore examined the association between systemic HIV-specific IFN- γ CD8 responses and viral load in the semen and blood of HIV-infected men. No correlation was observed between viral load in either semen or blood and systemic CD8 T-cell responses. Further studies of immune correlates of semen HIV shedding are needed.

HIV-specific CD8 T lymphocytes are critical in the host control of HIV replication. There is a clear temporal relationship between the development of HIV-specific CD8 T cells and viral control during acute HIV infection [1], and between experimental CD8 T-cell depletion and increases in the plasma viral load [2]. In addition, epitope-specific CD8 T cells place considerable evolutionary pressure on the virus in both primates and humans [3–8]. The magnitude of the systemic HIV-specific CD8 T-cell response has been inversely correlated with rates of disease progression [9] and plasma viral load in some studies [10–12], but not in others [13–15]. This may be because the inverse relationship only applies during early HIV infection [16–18], or to CD8 T cells recognizing specific HIV gene products, such as *Gag* [10].

The HIV plasma viral load is strongly associated with the probability of sexual HIV transmission [19], probably because the plasma viral load correlates with the amount of viral shedding in both semen [20–23] and female genital tract secretions [24–26]. HIV-specific

CD8 T cells can be detected in the semen [27] and the female cervix [28–30] of HIV-infected individuals. Furthermore, the specificity, function and ontogeny of HIV-specific CD8 T cells in the genital tract of infected individuals tend to reflect that found in the blood [31]. We therefore studied the relationship between systemic CD8 T cell responses and HIV shedding in the male urogenital tract.

Simultaneous blood and semen samples were collected from 20 treatment-naïve, chronically HIV-infected men enrolled through the Canadian Immunodeficiency Research Collaborative (CIRC), Toronto, Canada. Semen samples were collected by masturbation into a dry sterile container, and were immediately transported to the clinic at room temperature; blood was collected into acid citrate dextran. A first-void urine sample was screened for the presence of leukocytes using a dipstick (Bayer Diagnostics, Puteaux Cedex, France), and for infection by either *Neisseria gonorrhoeae* (NG) or *Chlamydia trachomatis* (CT) (Amplicor CT/NG assay, Roche Diagnostic, Quebec, Canada). Any participant with a positive result was excluded from analysis. Seminal plasma was isolated by centrifugation (~ 850 g/10 min), and peripheral blood mononuclear cells (PBMC) were obtained through Ficoll–Hypaque density centrifugation. The viral load in blood and seminal plasma was measured using the Versant HIV-1 RNA 3.0 assay (branched DNA; Bayer Diagnostics), as reverse transcriptase–polymerase chain reaction–based assays may be inhibited by semen constituents [32]. IFN- γ enzyme-linked immunospot assays were set up using PBMC at 1×10^5 per well, as previously described [33,34]. A matrix of 756 15-mer peptides overlapping by 11 amino acids, spanning the clade B HIV-1 genome (AIDS Research and Reference Reagent Program, Division of AIDS, NIAID, NIH) was established, with each peptide appearing uniquely in two separate pools at a final working concentration of 2 μ M. Responses were counted using an automated enzyme-linked immunospot counter (Cellular Technology Ltd., Cleveland, OH, USA). A positive HIV-specific CD8 response was defined as an HIV-specific response at least twofold higher than background (PBMC + 2 μ M DMSO), and 100 or more spot-forming units (SFU)/ 10^6 cells. All positive responses were confirmed using individual 15-mer peptides.

No PCR inhibition was evident when assaying the seminal plasma viral load. There was a trend towards an inverse relationship between the peripheral blood CD4 T-cell count (mean 574 cells/mm³, range 350–850 cells/mm³) and the HIV viral load in both semen and plasma ($P = 0.2$ for both). Viral loads in seminal and blood plasma were closely correlated ($r = 0.5$, $P = 0.03$; Fig. 1a), although viral levels in the blood were approximately 10-fold higher than in semen (mean 4.3 versus 3.3 log₁₀ copies/ml; $P < 0.001$,

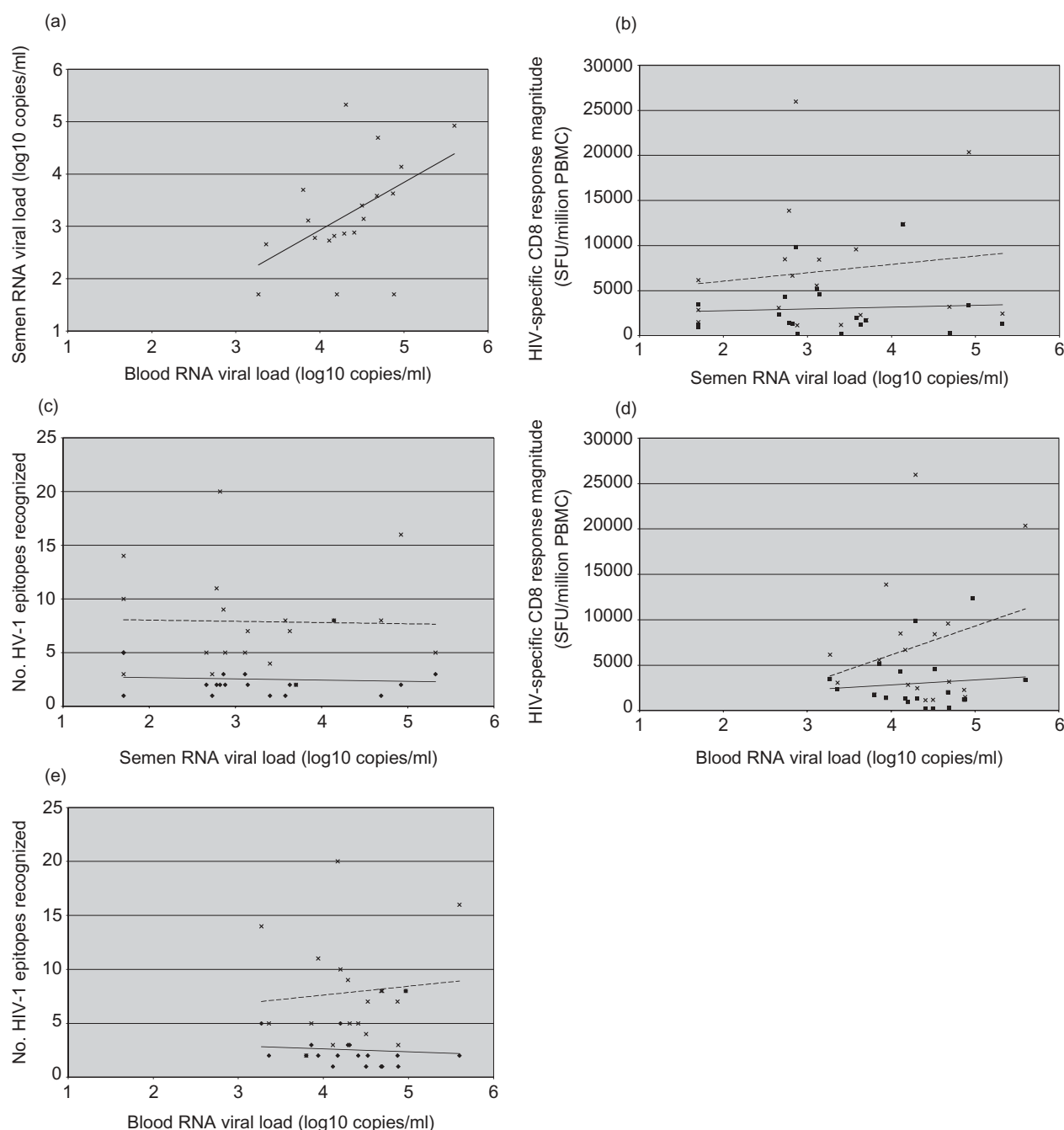


Fig. 1. Lack of association between semen or blood HIV-RNA viral load and systemic HIV-specific CD8 T-cell responses. There was a significant correlation between semen HIV-RNA shedding and blood RNA viral load ($r = 0.5$, $P = 0.03$) (a). Semen HIV shedding did not correlate with the magnitude of overall or *Gag*-specific systemic HIV-1-specific IFN- γ CD8 responses (b, dotted and solid lines of best fit, respectively), or with the number of epitopes recognized overall or within HIV-1 *Gag* (c, dotted and solid lines of best fit, respectively). Likewise, the blood viral load did not correlate with the magnitude of overall or *Gag*-specific systemic HIV-1-specific IFN- γ CD8 responses (d, dotted and solid lines of best fit, respectively), or with the number of HIV-1 epitopes recognized overall or within HIV-1 *Gag* (e, dotted and solid lines of best fit, respectively). See text for statistical analysis.

paired t -test). Study participants could be divided into three subgroups, based on their semen viral load; (i) three out of 20 (15%) had no detectable virus in the semen (≤ 50 copies/ml); (ii) three out of 20 (15%) had

high levels of HIV in the semen ($\geq 60\%$ blood viral load); and (iii) 14/20 individuals (70%) had detectable semen viral loads less than 60% of the blood viral load. In one individual, the semen viral load was 10-fold

higher than in blood (5.3 versus 4.3 log₁₀ copies/ml) in the absence of a detectable sexually transmitted infection or urethral inflammation.

Systemic CD8 IFN- γ responses were detected in all participants. A total of 159 epitopes were recognized (mean eight epitopes per participant, range two to 20 epitopes), and all were confirmed to be CD8 T-cell mediated by flow cytometry with intracellular cytokine staining. Responses focused on *Gag* (37% of epitopes), *Pol* (20%), *Nef* (20%), *Env* (13%), and less commonly on *Tat*, *Rev*, *Vpr*, *Vpu* and *Vif*. The mean total magnitude of CD8 T-cell IFN- γ responses for each participant was 6776 SFU/10⁶ PBMC (range 1135–25 955 SFU/10⁶ PBMC). Neither semen nor blood viral loads correlated with the total HIV-specific CD8 cell response magnitude (semen $r = 0.1$, $P = 0.6$; blood $r = 0.3$, $P = 0.3$) or the response breadth, defined as the total number of HIV epitopes recognized in an individual (semen $r = -0.03$, $P = 0.9$; blood $r = 1.0$, $P = 0.7$; Fig. 1). Although there was an inverse association between both the magnitude and breadth of *Tat*-specific responses and semen HIV shedding ($r = -0.5$ for both, $P = 0.02$; $P = 0.03$, respectively), only three out of 20 participants responded to *Tat*, limiting our power to draw firm conclusions, and significance was lost when correcting for multiple comparisons. No association was seen between the magnitude or breadth of *Gag*-specific CD8 T-cell responses and the viral load at either site (semen $r = 0.06$, $P = 0.8$; blood $r = -0.09$, $P = 0.7$; Fig. 1).

In conclusion, we have confirmed that there is a direct correlation between the HIV viral load in the semen and blood, that the viral load in semen tends to be approximately 10-fold lower than in blood, and that occasional 'super-shedders' [35] may pose a particular HIV-1 transmission risk [19]. Therefore, a better understanding of the immune correlates of viral shedding in semen will be important to inform the development of immunotherapeutics, topical microbicides and rational public health policy. However, we found no association between systemic HIV-specific CD8 T-cell responses and semen HIV shedding, or with the blood plasma viral load. In addition, no association was seen between *Gag*-specific systemic responses and viral levels in either semen or blood. These results strongly suggest that systemic HIV-specific CD8 T-cell IFN- γ responses are not directly associated with viral levels in the blood or semen compartments. Elucidating the immune associations of HIV semen shedding will be important in directing strategies to reduce sexual transmission.

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Impact of a treatment including tenofovir plus didanosine on the selection of the 65R mutation in highly drug-experienced HIV-infected patients

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Data from 20 highly drug-experienced HIV-infected patients receiving tenofovir plus didanosine as part of a salvage regimen were analysed. At baseline, all but one patient harboured a virus bearing at least one nucleoside excision mutation (NEM); in 13 cases (65%) three or more NEM were detectable. After a median of 26 weeks of treatment, two patients (10%) selected the 65R mutation. These results support the hypothesis that NEM hinder the selection of this mutation.

In vitro, reduced susceptibility to tenofovir disoproxil fumarate (tenofovir) can be detected in isolates with the 65R mutation, insertions at codon 69, or an increasing number of nucleoside excision mutations (NEM) [1,2]. The multidrug resistance 65R mutation was seldom (2–3%) detected in drug-experienced HIV-infected patients when tenofovir was added to the background failing regimen [3], but when it was combined with lamivudine and efavirenz in antiretroviral-naïve patients, the mutation was selected by 22% of failing cases [4]. Its incidence may be considerably higher (approximately 50%) in naïve patients failing on tenofovir plus abacavir or didanosine, and lamivudine [5–7]. One proposed explanation for these conflicting findings is an antagonism between the 65R mutation and NEM [8–10].

The aim of this study was to investigate the frequency of the 65R mutation in highly drug-experienced patients failing a regimen including tenofovir plus didanosine.

We used an observational database to select highly drug-experienced HIV-infected patients with all of the following characteristics: (i) HIV-RNA level greater than 1000 copies/ml before (within one month) and at least 20 weeks after starting a new regimen including tenofovir plus didanosine; (ii) an available genotype before (within one month) and at least 20 weeks after starting a new regimen including tenofovir plus didanosine; and (iii) no change in the antiretroviral regimen between the first and second resistance test.

Genotype was performed by direct sequencing, using Megabace 1000 (Pharmacia-Amersham, Freiburg, Germany). The HIV-RNA level was assessed by means of nucleic acid sequence-based amplification (Nuclisens HIV-1 Bio-Merieux, Boxtel, the Netherlands); the quantification limit was 80 copies/ml.

The database of the Infectious Diseases Clinic of Vita-Salute, Salute San Raffaele University currently contains the results of 903 HIV genotypes and clinical data relating to 292 patients currently receiving a tenofovir-including regimen. Twenty patients fulfilled the inclusion criteria. The nucleoside analogues associated with tenofovir and didanosine included stavudine, zidovudine or lamivudine in four, none, and 11 cases, respectively. Ten patients received a combination of three nucleos(t)ide reverse transcriptase inhibitors.

At baseline, CD4 T lymphocytes were 198 [interquartile range (IQR) 143–265]/ μ l and HIV-RNA 4.91 $\log_{10}$ copies/ml (4.53–5.23). The median duration of antiretroviral treatment was 7.9 years (5.7–8.7), and

the median number of reverse transcriptase mutations was 5.5 (three to eight). All but one patient harboured a virus bearing at least one NEM; in 13 cases (65%), three or more NEM were detectable. Ten patients had the 41L+210W+215F/Y complex and three the 67N+70R+219E/Q complex. The median (IQR) number of NEM was three (two to four). None of the patients studied showed the 65R mutation, whereas six (30%) harboured a variant carrying the 74V substitution.

After a median of 26 weeks of treatment (24–34), CD4 T lymphocytes were 148 (100–297)/ μ l and HIV-RNA 4.57 $\log_{10}$ copies/ml (4.11–4.88). Two patients (one also receiving lamivudine and the other also receiving nevirapine) selected the 65R mutation. One patient receiving tenofovir plus didanosine and boosted amprenavir selected the 151M and the 115F mutation, and one receiving tenofovir plus didanosine and lamivudine selected an insertion at codon 69. The median (IQR) number of NEM was four (two to four) (Fig. 1).

Despite the small sample size, this study of patients receiving a tenofovir-including regimen considered at high risk for the selection of the 65R mutation, allowed us to show that this event may be less frequent in a given clinical setting.

Ten out of 20 naive HIV-infected patients (50%) failing a combination of tenofovir, didanosine and lamivudine selected variants bearing this amino acid substitution 16 weeks after starting their first antiretroviral regimen [7].

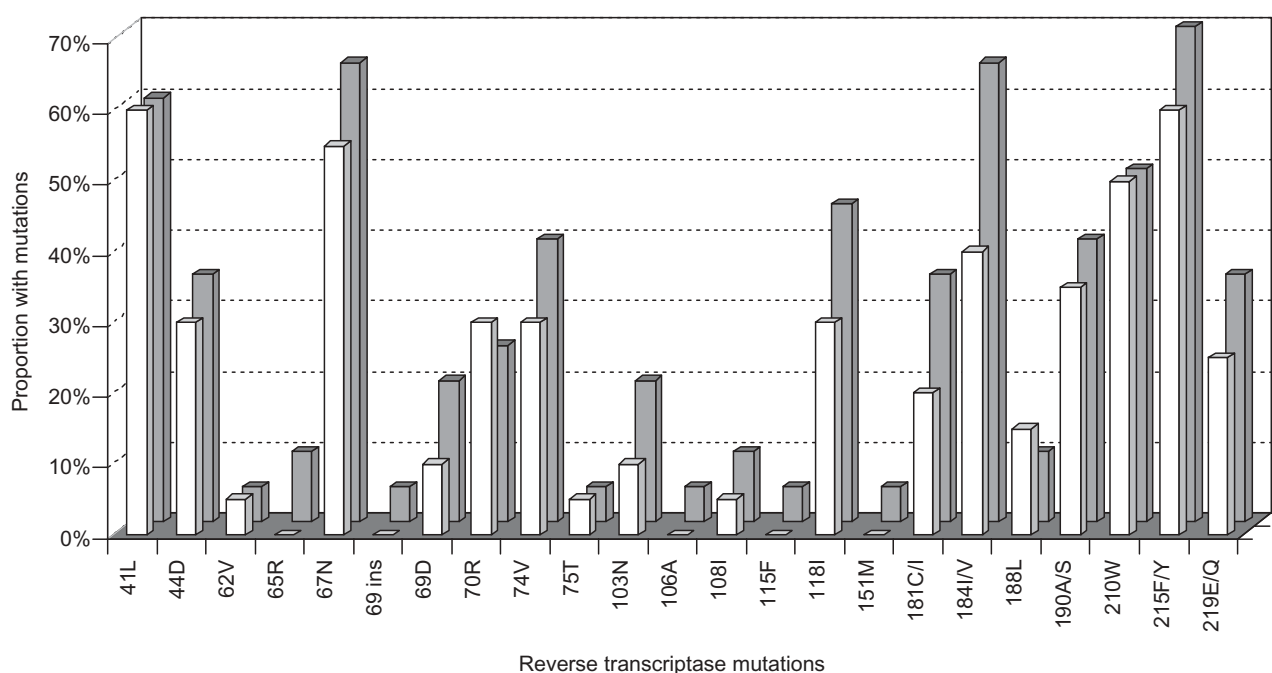


Fig. 1. Proportion of patients with mutations related to resistance to reverse transcriptase inhibitors before and after a median of 26 weeks' treatment with a regimen including tenofovir plus didanosine. White bars: baseline; grey bars: follow-up.

All of the variants bearing the 65R mutation also bore the 184V mutation. Only 10% of our patients selected 65R mutants after a median of 26 weeks, despite failure on a regimen including tenofovir plus didanosine. As selection of the 65R mutation has been observed within 16 weeks in naive patients treated with tenofovir plus didanosine, it is unlikely that we underestimated its rate of occurrence.

Retrospective analyses have shown that individuals receiving tenofovir and a thymidine analogue (zidovudine or stavudine) are significantly less likely to develop the 65R mutation [11], whereas the selection of 65R variants are favoured by regimens not including thymidine analogues [12,13]. As only 20% of our patients received stavudine (and none zidovudine) in association with tenofovir and didanosine, a high rate of 65R mutants could have been expected.

Fifty per cent of our patients received lamivudine in association with tenofovir and didanosine. In the study described by Jemsek *et al.* [7], the 65R variants developed in patients receiving tenofovir, didanosine and lamivudine. The smaller number of patients treated with lamivudine might therefore explain our different findings, but data from a large database have revealed that exposure to lamivudine reduces the risk of developing the 65R mutation [14].

We suggest that the low prevalence of 65R mutants in our study may be because of the high prevalence of NEM at baseline. Baseline genotyping revealed at least one NEM in all but one patient, with 65% harbouring a variant bearing three or more NEM. Although tenofovir promotes the selection of the 65R mutation, zidovudine might contribute to its deselection [8]. Bidirectional phenotypic antagonism between this mutation and NEM has been shown [9], as has a decreased excision of nucleoside reverse transcriptase inhibitors in the presence of 65R mutants [10]. Winston *et al.* [11] reported that the fewer NEM present at baseline, the more likely the 65R variant was to develop at the time of the failure of regimens not necessarily including didanosine or abacavir in combination with tenofovir. Similarly, other retrospective studies have never detected the 65R mutation together with reverse transcriptase substitutions at codon 215 [15,16]. Overall, these data indicate antagonism between NEM and the 65R mutation, and thus provide a likely explanation for our findings.

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Comparison of point-of-care rapid HIV testing in three clinical venues

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Rapid HIV testing with same-visit results should increase the number of individuals who know they are HIV infected. We assessed the acceptability and feasibility of point-of-care rapid testing in three public venues, a sexually transmitted disease clinic, a county jail, and an emergency department. Over 98% of all participants received their results, and 82% of newly identified HIV-positive participants entered care. Point-of-care rapid testing was feasible, acceptable, and may improve entry into care.

The 1–2 week turnaround time for conventional HIV testing has been a barrier to voluntary screening and patient receipt of results. Rapid HIV tests create opportunities to expand testing venues and provide same-visit results. To assess patient acceptance, feasibility of point-of-care testing, and impact on entry into care, we studied rapid HIV testing at the CORE Center Sexually Transmitted Disease (STD) Clinic [1]. In this report, we compare that experience with rapid HIV testing in two other high-prevalence venues, Cook County Jail's female intake area, which processes 15 000 women detainees per year (50% released within 36 h), and Stroger (Cook County) Hospital's Adult Emergency Department (ED), which has approximately 120 000 visits per year.

Individuals aged 18 years and older were eligible if they spoke English or Spanish, were not known to be HIV infected, had not been tested for HIV within 3 months, and evidenced mental stability to receive same-visit HIV results. Participants provided written informed consent for the research protocol and for HIV testing.

The rapid HIV test used was the SUDS (Abbott/Murex, Norcross, GA, USA) assay, the only US Food and Drug Administration-approved rapid HIV test available at the time. Details of the procedures are described elsewhere [1]. In brief, three health educators offered testing to eligible participants, obtained consent, collected blood, performed the test, provided partici-

pants with negative or preliminary positive results [2], and scheduled newly diagnosed HIV-infected participants for follow-up at the CORE Center HIV clinic within 2 weeks.

All SUDS tests were supplemented with a conventional enzyme immunoassay. A Western blot test was performed on repeatedly reactive SUDS or enzyme immunoassay reactive tests. The availability of the SUDS test determined the study duration at each site.

The logistics at each site affected the timing of participant recruitment. In the STD clinic, patient flow was re-arranged to allow pretest counseling and phlebotomy before a provider saw the patient [1]. In the jail female intake area, the study staff approached the detainees in a corner of a large room, in open view of security officers, before jail medical screening. Detainees with reactive SUDS tests were told the results in a confidential area. In the ED, study staff approached eligible patients who were not in a critical condition, after ED registration or at the bedside.

Computer records and medical charts were reviewed within 24 h of the first clinic appointment and at 6 months. Outreach investigation was initiated for any HIV-positive participant who failed to keep the initial appointment. Retention into care was defined as two or more visits within the 6 months.

Rapid HIV testing was accepted by 29–69% of participants. Although the STD clinic had the highest acceptance rate (Table 1), point-of-care testing was feasible at all three test venues. Ineligibility, 20–30%, mostly resulted from claims of recent HIV testing. Of the ineligible individuals, those at the jail and ED were more likely than those in the STD clinic (8.4, 8.2, and 3.3%, respectively) to be refused for psychiatric or substance abuse reasons.

HIV infection rates were 0.9–2.8% (Table 1). All participants with reactive rapid tests and over 98% of the others received results within 2 h. The demographics of the HIV-positive patients were similar to the populations tested at each site. More than 75% of positive tests were in 25–44 year olds, and 46–78% were in heterosexuals who reported no risk factors. Overall, 82% of participants entered into medical care; the majority had initial CD4 T-cell counts of less than 350 cells/mm³.

In Chicago's busiest ED, the rapid test process was logistically feasible and surprisingly smooth. Despite having the lowest participant acceptance (29%), the large ED provided the greatest number of newly diagnosed cases. Based on a subsequent study in our ED in which the acceptance of rapid testing was 60% [3], the

Table 1. Comparison of test acceptance, patient characteristics, and results at three rapid HIV test sites.

Variable	Site ^a		
	STD clinic	Jail	ED
Duration of study (months)	10	4.5	8.5
Test acceptance and eligibility, N (%)			
Patients approached	2641	3048	7072
Patients eligible	1977 (75)	2128 (70)	5668 (80)
Patients accepted rapid testing	1372 (69)	988 (46)	1652 (29)
Participant characteristics, N (%)	HIV+/all participants		
Sex			
Male	26 (70)/878 (64)	0/0	38 (83)/885 (54)
Female	11 (30)/494 (36)	9/988	8 (17)/767 (46)
Race/ethnicity			
African American	26 (70)/1014 (74)	6 (67)/776 (79)	37 (81)/1216 (74)
Hispanic	7 (19)/169 (13)	3 (33)/74 (7)	7 (15)/251 (15)
White	1 (3)/133 (10)	0/121 (12)	1 (2)/127 (8)
Other	3 (8)/47 (3)	0/17 (2)	1 (2)/58 (3)
Age (years)			
18–24	2 (5)/407 (30)	0/209 (21)	3 (7)/343 (21)
25–44	29 (79)/802 (59)	8 (89)/686 (70)	35 (76)/1025 (62)
≥ 45	6 (16)/163 (1)	1 (11)/92 (9)	8 (17)/280 (17)
Missing	0	1	4
Risk factor, N (%) (not mutually exclusive)			
Men sex with men	10 (27)/39 (3)	0/0	14 (30)/20 (1)
Sex partner HIV infected	6 (16)/66 (5)	0/4 (< 1)	3 (7)/25 (3)
Bisexual	5 (14)/39 (3)	2 (22)/55 (6)	0/38 (2)
Intravenous drug use	2 (5)/9 (0.7)	0/11 (1)	6 (13)/18 (1)
Sex partner's intravenous drug use	1 (3)/17 (1)	0/25 (3)	3 (7)/43 (2.6)
No identified risk factor	17 (46)/1176 (86)	7 (78)/858 (87)	22 (48)/1498 (91)
Results, N (%)			
Patients received result	1357 (98.9)	985 (99.7)	1640 (99.3)
Positive rapid HIV test (newly identified HIV)	37 (2.7)	9 (0.9)	46 (2.8)
New HIV+ entered into care	36 (100) ^b	2 (22)	36 (80)
New HIV+ retained in care ^c	30 (83)	0	31 (86)

^aThe Cook County Bureau of Health Services, Chicago IL: STD clinic, CORE Center Sexually Transmitted Disease Clinic, October 1999–August 2000; Jail, Cook County Jail – Female Intake Area, October–December 2000, April–May 2001; ED, Stroger (Cook County) Hospital Emergency Department, June 2001–February 2002.

^bOne participant died before the first visit.

^cAt least one additional provider visit within 6 months of initial visit.

potential explanations for the 29% acceptance rate included interpersonal skills of the different study staff, a lack of bilingual study staff, and the inclusion of older participants (up to age 60 years) in the present study.

The jail female intake provided a confined audience, but less than half of those eligible accepted testing. Because HIV testing in the intake area was new, we required strong administrative support to ensure cooperation from the medical and security staffs, whose primary goals were prompt triage and the maintenance of order. To our knowledge, there is no literature on rapid HIV testing in correctional facilities. A review of conventional test results collected in 1992–1998 at correctional facilities [4] reported that 3.4% of over 500 000 tests were positive, 56% in newly identified infections. A study among Texas correctional system's new inmates [5] showed a 2.5% HIV prevalence in female state jail detainees, a finding similar to our prevalence of 2.3%

(0.9% rapid test positive and 1.4% self-reported HIV positive).

Studies of rapid HIV tests have not systematically evaluated entry into care [1,6,7]. Most of our ED and STD clinic, but few jail participants, entered care. Although this may suggest that the jail testing venue was not productive, the reasons for this finding need further study.

There are two important limitations to the generalizability of our results. First, we used dedicated, rather than indigenous staff to perform counseling, testing, and result reporting. By using existing providers, it may be possible to approach more individuals in large venues such as the ED. Second, until recently, the only US Food and Drug Administration-approved rapid HIV test was SUDS, which is categorized by Clinical Laboratory Improvement Amendments as moderately complex [8]. Three recently approved, new rapid HIV

tests [8–11] are user friendly and one is Clinical Laboratory Improvement Amendments-waived; they afford point-of-care testing and should only improve the feasibility, turnaround time, and use of existing staff.

In conclusion, we found that point-of-care rapid HIV testing was logistically feasible and acceptable to many participants and staff in three very different venues. All positive participants learned their results and most entered into care. The use of rapid HIV testing at similar sites may facilitate testing for many patients who do not perceive themselves at risk or who do not otherwise access medical care.

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CD4 cell recovery during successful antiretroviral therapy in naive HIV-infected patients: the role of intravenous drug use

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We evaluated the impact of HIV risk practice on immune reconstitution in a prospective cohort of 288 patients (176 former injecting drug users) who maintained complete virus suppression for more than 24 months. Significant differences in CD4 cell counts at 6 and 24 months were detected. Multivariate analysis showed that drug use was an independent predictor of poor immunological recovery. Injection drug abuse impairs short and long-term CD4 cell recovery in HIV-positive patients initiating successful highly active antiretroviral therapy.

Since the introduction of highly active antiretroviral therapy (HAART), a dramatic improvement in the life expectancy of HIV-1-infected patients has occurred [1]. However, many patients who were former injecting drug users (IDU) have co-infection with HIV-1 and hepatitis C virus (HCV) and their immunological recovery could be impaired. The consumption of some drugs has been proved to be a risk factor that alters the normal immune function [2–6], and could be involved in the lack of immunological response in patients treated with HAART [5,6]. We assessed the impact of intravenous drug use on the immunological outcome of a group of antiretroviral-naïve HIV-infected patients who began receiving HAART and had long-term suppression of HIV-1 RNA.

All antiretroviral-naïve HIV-seropositive patients ($n = 501$), who had initiated HAART in a tertiary centre located in Madrid (Spain) since 1996 were included. For the purpose of analysis, we selected HIV-infected individuals ($n = 288$) who had maintained complete viral suppression for more than 2 years, and had available baseline CD4 cell counts and serological assessment of HCV or hepatitis B co-infection. Demographics and baseline characteristics can be observed in Table 1. Antiretroviral response was defined as an HIV-RNA level of less than 50 copies/ml. The main outcome variable was defined as an increase in CD4 cell counts of more than 50 cells/ μ l after 24 months of follow-up. Immunological responses were assessed at months 6, 12, 18 and 24, and were analysed according to the HIV risk practice. Secondary clinical endpoints

Table 1. Baseline demographic and clinical characteristics of 288 naive HIV-infected patients, according to HIV-acquisition risk practice, who had complete virus suppression while receiving highly active antiretroviral therapy and who had completed at least 2 years of follow-up.

	IDU-negative (n = 112)	IDU-positive (n = 176)	P
Mean age (years)	38	34	0.028
Sex male	76 (68%)	138 (78%)	0.046
Previous AIDS diagnosis	31 (28%)	53 (30%)	0.658
Co-infection			
HBV	8 (7%)	10 (6%)	0.590
HCV	18 (16%)	167 (95%)	0.000
Median CD4 cell count, $\times 10^6/l$	258	214	0.413
Median HIV-RNA level, copies/ml	5.07 log ₁₀	4.87 log ₁₀	0.106
Initial therapy with a PI-based regimen	91%	91%	0.905

HBV, hepatitis B virus; HCV, hepatitis C virus; IDU, injecting drug user; PI, protease inhibitor.

were death and new clinical events. Multiple regression logistic models with a backward modeling strategy were used to assess the association of intravenous drug use with the main outcome variable, after adjusting for other confounding factors. We adopted a 5% significance level for all tests. Analyses were performed by using SPSS (version 11.0; Chicago, IL, USA).

A total of 176 patients (61%) had acquired HIV-1 infection through intravenous drug use. These individuals had a significantly worse CD4 cell count recovery, which remained significantly different through all the study period. The mean CD4 cell counts in the intravenous and non-intravenous subgroups of patients, at months 6, 12 and 18 were: 341 versus 456 ($P = 0.037$), 396 versus 490 ($P = 0.05$), and 440 versus 559 ($P = 0.024$) $\times 10^6/l$, respectively. After 24 months, data from 257 patients were available for analyses. The CD4 cell count was significantly higher in patients who acquired HIV infection by routes other than intravenous drug use (588 cells $\times 10^6/l$ versus 472 cells $\times 10^6/l$; $P = 0.013$). Multivariate analysis showed that only the acquisition of HIV-1 through intravenous drug use remained as an independent predictor of the CD4 cell count increase at 24 months. Former IDU were less likely to increase their CD4 cell counts more than 50 cells/ μl than non-IDU [odds ratio (OR) 0.23; 95% confidence interval (CI) 0.076–0.717]. Clinical progression was uncommon. A total of 51 HIV-related clinical events occurred, mainly herpes zoster ($n = 21$, 16 in IDU), pulmonary/disseminated tuberculosis ($n = 7$, four in IDU), invasive cervical carcinoma ($n = 2$), and progressive multifocal leukoencephalopathy ($n = 2$). Overall, six patients (2.3%) died (five in the IDU group; $P = 0.41$).

A number of factors that may influence immune recovery in patients on HAART have been identified. One of the most controversial issues has been the impact of HCV co-infection in the course of HIV disease. In our series, intravenous drug use and not

HCV co-infection determined the amount and speed of the increase in CD4 cells counts. Greub *et al.* [7] found an independent association of HCV co-infection with a worse clinical outcome. Smaller increases in the CD4 cell count were observed in HIV-1/HCV co-infected patients. Almost 90% of HCV-infected patients were IDU, and former and active use were not precisely differentiated. These facts could have produced an over-adjustment of the importance of HCV co-infection on the immunological outcomes. They concluded that both HCV co-infection and active intravenous drug use could be important co-factors in morbidity and mortality among HIV-1-infected patients. In our series, only patients who were antiretroviral naive, former (non-active) IDU, initiated HAART with a good adherence, and had a successful suppression of HIV-1 RNA after 24 months of follow-up were included. This subgroup population seems to represent the most appropriate group to assess the effect of different co-factors on immune reconstitution. We focused mainly on immunological recovery, and observed that former IDU had approximately a fourfold greater risk of a poor immunological recovery than non-IDU (OR 0.23; 95% CI 0.076–0.717). This over-risk remains significant after adjustment for other conditions such as PI-based regimens and baseline viral load.

The relationship between illicit drug use and the immune system has previously been established and supports our findings. Opiates and cocaine can suppress the functional activities of human peripheral blood mononuclear cells, which are known to play a key role in host defence against intracellular opportunistic pathogens [8–10]. Apoptosis has been shown to be an important mechanism of T-cell depletion in HIV infection, independent of viral replication. The activation of immune cells correlates with CD4 T-cell apoptosis and is a stronger marker of HIV-1 disease progression than HIV-RNA levels [8–10]. A decrease in the rate of lymphocyte apoptosis occurred after the

initiation of HAART, and improved T-cell survival, increased T-cell production, and a decreased rate of ex-vivo apoptosis can be achieved [10]. Morphine has been shown to alter DNA repair in human lymphocytes by a variety of mechanisms [11]. Opiate use *in vivo* and *in vitro* reduces the ability of human peripheral lymphocytes to repair DNA damage. It leads to increased genetic damage in the cell and to increases in the host cell mutation rate and the rate of apoptosis [11]. This mechanism can give further support to the blunt CD4 cell recovery observed among our former IDU.

There are several limitations to our study. It was an observational, clinical cohort study, and at the moment of inclusion some characteristics of the HIV-1-infected population were not well balanced. The number of patients included is relatively small and therefore our multivariate analyses was limited to a reduced number of variables. Finally, we cannot exclude the possibility that our results were distorted by residual confounding or unmeasured factors.

In summary, a long-term poorer increase in the CD4 cell count despite a successful virological suppression is more frequently observed in naive HIV-1-infected patients who acquired their infection by injecting drug use. Whether the findings of our study are relevant to the management of HIV-1 infection remains to be seen. Further studies are needed to confirm these observations, to obtain a better understanding of the complex interaction between opiates, HIV-1 and the immune system, and to assess more effective therapeutic measures in this population.

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