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AIDS 2009, **23**:1443–1451

Detectable cerebrospinal fluid HIV RNA with associated neurological deficits, despite suppression of HIV replication in the plasma compartment

Highly-active antiretroviral therapy (HAART) rapidly suppresses HIV replication in plasma; however, concerns exist over the ability of antiretrovirals (ARVs) to penetrate and suppress viral replication in biological compartments such as the central nervous system (CNS) and genital tract [1,2]. Ongoing cerebrospinal fluid (CSF) HIV replication in the presence of virological suppression in the plasma compartment by HAART is not well described [3]. We describe the case of an individual treated with HAART, successfully maintaining plasma HIV RNA below 50 copies/ml, who had ongoing CSF HIV replication with neurological symptoms.

A 33-year-old white man presented with left leg weakness affecting his gait. An HIV test 6 months earlier was negative. Neurological examination demonstrated increased tone, sustained ankle clonus, pyramidal distribution weakness (Medical Research Council grade 4/5), brisk reflexes and an extensor plantar response affecting the left leg, with normal findings elsewhere.

Cerebral MRI revealed a nonenhancing right precentral gyrus lesion (Fig. 1). CSF contained 13 mononuclear cells/μl, protein 0.4 g/l, glucose 3.6 mmol/l (serum 5.2 mmol/l), and viral PCRs (John Cunningham virus, herpes simplex virus, varicella-zoster virus, Epstein–Barr virus, enterovirus and cytomegalovirus) were negative, as were CSF tests for cryptococcal antigen, syphilis and culture for bacteria, fungi and tuberculosis. Investigations found no obvious cause for a cerebrovascular event. Serum was positive for HIV-1 antibody, CD4 cell count was 190 cells/μl (27%), plasma HIV viral load was 361 837 copies/ml and human leukocyte antigen-B5701 allele test was negative. Genotypic analysis of plasma revealed wild-type virus (clade B) [4].

A clinical diagnosis of recent HIV seroconversion with neurological involvement was made. The patient was commenced on HAART [abacavir, lamivudine (Kivexa) and saquinavir/ritonavir], and plasma HIV RNA of less than 50 copies/ml was achieved by week 12. Follow-up cerebral MRI was unchanged 3 and 6 months later.

Twelve months later, the patient experienced worsening of left leg weakness. HAART was unchanged, plasma HIV RNA was consistently less than 50 copies/ml and CD4 cell count had risen to 450 cells/μl (47%). MRI revealed additional diffuse-enhancing lesions throughout both cerebral hemispheres. CSF white cell count was four per microliter, and the CSF HIV RNA level was 718 copies/ml.

Two months later, plasma HIV RNA remained less than 50 copies/ml, and CSF HIV RNA was 676 copies/ml. CSF sequencing of reverse transcriptase/polymerase confirmed clade B HIV-1 virus, and M184V and D67E were observed. Furthermore, the CSF on this occasion contained 44 mononuclear cells/μl, protein 0.53 g/l and glucose 3.2 mmol/l (serum 5.2 mmol/l). Tuberculosis culture and all other CSF tests were negative.

HAART was switched to abacavir, zidovudine, lamivudine (Trizivir) and nevirapine [200 mg twice daily (b.i.d)] with the aim of improving CNS penetration of ARV agents. MRI performed 3 months later showed that the prefrontal gyrus lesion was unchanged; however, the small enhancing lesions had resolved. The CSF pleocytosis had fully resolved, and CSF HIV viral load was now less than 50 copies/ml.

The case demonstrates the ability of HIV to continue replicating within the CNS compartment, despite the plasma viral load being suppressed by HAART to below 50 copies/ml. This phenomenon has not previously been well described. Along with other investigations, CSF HIV RNA quantification and genotype should be considered early in any individual with neurological symptoms, irrespective of plasma HIV RNA level.

Further research will establish optimal ARV combinations for such individuals and demonstrate whether longitudinal monitoring of CSF RNA and genotype (with HAART adjustment accordingly) can benefit clinical outcome. The work conducted by Letendre *et al.* [5] demonstrated the use of a CNS penetration efficacy ranking score (based on available data of ARV chemical properties, concentration in the CSF and/or results in clinical studies) that is associated with a lower level of CSF HIV RNA. Long-term clinical outcome data for this strategy, however, do not yet exist.

Of interest, our patient developed a late CSF pleocytosis without evidence of additional CNS infections, and after changing HAART, this resolved. It is possible that CNS inflammation developed in response to persistent HIV replication and resolved with 'CNS-optimized' HAART. This mechanism maybe similar to the brief CSF

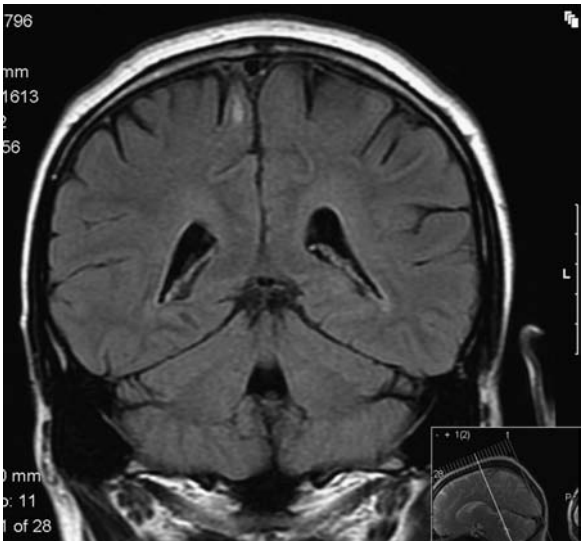

Fig. 1. T2-weighted flair image demonstrating enhancing lesion in the right precentral gyrus.

pleocytosis demonstrated in approximately half of the patients on stopping therapy [6].

The shortcomings of this report include that ultrasensitive techniques were not used for HIV RNA quantification, and minority level resistance mutations may have been present in plasma at baseline. Furthermore, CSF HIV RNA and genotype were not performed at baseline and, therefore, it cannot be confirmed that the CSF HIV resistance mutations that developed were due to the therapy administered.

Clinicians managing individuals with neurological symptoms and an undetectable plasma HIV RNA should be aware of the possibility of CSF virological replication and the selection of viral variants associated with drug resistance.

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Are the effects of isoniazid preventive therapy and highly active antiretroviral therapy additive in preventing HIV-associated tuberculosis?

Golub *et al.* [1] recently reported a retrospective analysis of rates of incident tuberculosis (TB) in a large observational cohort of 2778 patients accessing HIV care in rural and urban South Africa. The TB incidence rate was highest [7.1/100 person-years; 95% confidence interval (CI) = 6.2–8.2] during the period of care when patients did not receive isoniazid preventive therapy (IPT) or highly active antiretroviral therapy (HAART). The rates were lower during person-time that accrued throughout follow-up after initiation of IPT (5.2/100 person-years; 95% CI = 3.4–7.8) and throughout follow-up on HAART alone (4.6/100 person-years, 95% CI = 3.4–6.2). The rate was lower still (1.1/100 person-years 95% CI = 0.2–7.6) during person-time accrued during sequential IPT and HAART (IPT + HAART). The authors concluded that TB risk was significantly reduced by IPT in HAART-treated adults. It was further concluded that 'the dramatic reduction in TB risk' demonstrated in this study together with supportive data from a similarly analysed study from Brazil [2] indicates that widespread use

of IPT should be implemented in conjunction with the roll-out of HAART.

First, it is notable that HAART alone but not IPT alone was associated with significantly lower TB incidence rates compared with the nonintervention group in both adjusted and unadjusted analyses. In addition, no significant difference was observed in the incidence rates comparing the HAART-only and the IPT + HAART groups. Thus, these analyses do not demonstrate a significant additive effect in TB prevention by treating patients with both IPT and HAART.

In further analysis, Kaplan–Meier survival estimates appeared to support a significant difference between TB-free survival in the IPT + HAART group and all other treatment groups. Surprisingly, however, the Kaplan–Meier analysis showed that no TB events occurred among patients in the IPT + HAART group during the first 3 years of observation despite low CD4 cell counts (median, 176 cells/μl at the time of HAART initiation). We are concerned that these conclusions may have been erroneously drawn as a result of analysis of groups with significant selection bias.

Among 62 patients within the IPT + HAART group, 61 had initiated IPT for a median of 1.0 years [interquartile range (IQR) = 0.5–1.9] before starting HAART. In this analysis, person-time of observation was censored when patients developed TB. Thus, only selected patients who remained alive and free of TB during the period between IPT initiation and HAART initiation would be eligible for inclusion in the IPT + HAART group. Thus, the TB incidence rate during the median of 1.0 years (IQR 0.5–1.9) of observation before HAART would have been inappropriately assessed as zero cases/100 person-years. However, the IPT + HAART group had markedly lower CD4 cell counts than the IPT-alone group and therefore the true incidence rate would very likely have exceeded 5.2 cases/100 person-years in the pre-ART period, potentially adding several more cases to the single case actually reported in this treatment group. Furthermore, no deaths were reported in this survival analysis and additional cases of TB may well have remained unascertained among those who died. Inclusion of such additional TB cases would likely have negated the apparent observed group differences in the Kaplan–Meier analysis.

The benefits of IPT in HIV-infected patients included in randomized controlled trials have been clearly demonstrated [3,4]. However, the lack of significant effectiveness of IPT alone in this South African study and in the large Brazilian cohort study [2] raises concerns about the effectiveness of this intervention in routine clinical practice. Potential explanations for lack of IPT efficacy may include poor adherence and the inclusion of tuberculin skin test-negative individuals. However, in both these studies, the strongest predictor of TB incidence rates was the baseline CD4 cell count, with 60–90% lower rates in those with CD4 cell strata above 200 cells/μl. Furthermore, there are no data from randomized controlled trials that specifically demonstrate the efficacy of primary IPT at low CD4 cell counts. We wonder, therefore, whether the inclusion of significant numbers of individuals with CD4 cell counts under 200 cells/μl may partially explain the apparent lack of a statistically significant reduction of TB rates during and after IPT.

In view of the clear benefits of HAART on survival and TB incidence [5], it would be unethical to perform studies of 6 months IPT or placebo before initiating HAART in eligible patients. TB rates during early HAART are very high and related to the baseline CD4 cell count at treatment initiation, remain high in those with suboptimal CD4 cell recovery and continue to be elevated even in those with optimal CD4 cell recovery [6–8]. There remains an urgent need to demonstrate an additive benefit of IPT over and above CD4 cell recovery after initiation of HAART and to identify the optimal timing of IPT before this can be scientifically justified as an integrated component of HAART rollout.

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Additive effects of isoniazid preventive therapy and HAART

Our thanks to Wood *et al.* [1] for their interest in our study describing the benefits of isoniazid preventive therapy (IPT) and highly active antiretroviral therapy (HAART) for HIV-infected patients being followed prospectively in South Africa [2]. Tuberculosis is an exceedingly common opportunistic infection and the leading cause of death in HIV-infected adults, irrespective of their HAART treatment status. Given that the effectiveness of IPT has been clearly demonstrated in multiple trials and cohort analyses [3] but its uptake incredibly poor in resource-limited settings [4], we were interested in understanding the impact of both HAART and IPT in a clinical cohort in South Africa, a very high-burden area. As clearly stated in our study, clinical cohort studies are subject to a number of limitations and potential biases that are not present in randomized trials. Conversely, however, clinical cohorts represent a much more real life situation than clinical trials, in which patients are not excluded because of comorbid conditions and adherence to protocols is imperfect. We endeavored to learn the most from our data by constructing analyses that minimized potential biases and adjusted for important covariates, including time and CD4 cell counts. We believe that Wood *et al.* [1] may have misunderstood some of these analytical methods in their critique of our results.

First, we were careful to categorize all follow-up time into four categories according to exposure to IPT and/or HAART: treatment naive, IPT only, HAART only, and both IPT and HAART – the majority of whom received IPT prior to receiving HAART. Thus, we compared the incidence of tuberculosis during treatment-naive follow-up with the incidence in each of the different treatment categories and did not use time accrued in a different category in our calculations. For example, the patients who received both IPT then HAART (without being censored) contributed person time to three categories (treatment naive, IPT only, and IPT/HAART), and any tuberculosis event would be attributed to the IPT/HAART time period. The simple rate calculations based on these person-years can be affected by survival bias, with person-years in one exposure group tending to be earlier during follow up than those exposed to both. For the Kaplan–Meier and Cox models, however, all patients followed a time-since-cohort entry timeline, with staggered entry according to category transitions. Thus, for example, a patient who has been followed for exactly 2 years and is on both IPT and HAART is compared at that instant with all the rest who also have survived (not been censored) at least 2 years. We did not directly compare patients receiving HAART with those receiving both IPT and HAART; the incidence within each of these time periods was compared with the incidence among treatment-naive patients. What our data show, therefore, is that the point estimate of tuberculosis incidence for those who received both IPT and HAART is considerably lower than for those who received either intervention alone, suggesting an interaction between these two interventions.

As noted by Wood *et al.* [1], CD4 cell counts are important potential confounders in an analysis such as ours, and we adjusted for baseline CD4 cell counts accordingly. The median CD4 cell count at time of IPT for everyone receiving IPT, including those who later received HAART, was 399 cells/μl. Patients who received HAART following IPT had a median CD4 cell count of 176 cells/μl at HAART initiation, similar to those patients who received HAART and no IPT (median CD4 cell count, 145 cells/μl). Our adjusted Cox proportional hazards model revealed that patients receiving both IPT and HAART had a significantly reduced risk of tuberculosis after adjustment for baseline CD4 cell count (89% reduction). The protective effect of HAART only compared with treatment-naive patients was significant as well (64% reduction). As stated above, these estimates cannot be directly compared in our analysis, but clearly suggest that patients receiving both therapies have greater protection.

Wood *et al.* [1] also wonder whether IPT alone was not significantly associated with protection from tuberculosis because of the inclusion of many patients with CD4 cell counts less than 200 cells/μl. When we examined only patients with baseline CD4 cell count more than

200 cells/μl, the effectiveness of IPT was only slightly better than in our overall model [adjusted hazards ratio (aHR) = 0.81; 95% confidence interval (CI) = 0.48–1.38 versus overall aHR = 0.87; 95% CI = 0.55–1.36].

As noted by Wood *et al.* [1], the benefits of IPT have been clearly established, and its use is recommended by the WHO. Although it is also clear that HAART reduces the risk of tuberculosis, rates are more than 10-fold higher than in HIV-uninfected individuals, and our data suggest that IPT can further reduce this risk. Although it is reasonable to study whether IPT significantly improves protection compared with HAART alone in patients with advanced HIV infection, we do not believe that it is appropriate to withhold IPT from patients in HAART roll-out programs pending the results of studies. By analogy, when HAART was first introduced and its benefits were being appreciated, guidelines for prevention of opportunistic infections remained in place, including the use of cotrimoxazole and azithromycin. Only when controlled trials demonstrated that these therapies were not necessary following a response to HAART did the guidelines change. If clinical trials were to show that there is no additional benefit to IPT in patients receiving HAART (and we are skeptical of this), then Wood *et al.* [1] would be justified in recommending that IPT be omitted from HAART treatment programs. In the meanwhile, ignoring the results of controlled trials with more than 11 000 participants and the results of cohort studies with approximately 15 000 participants [2,5] showing the benefits of IPT means that millions of patients are exposed to an unjustified risk.

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Idiopathic central pontine myelinolysis in an eunatremic patient with AIDS

Central pontine myelinolysis (CPM) is an uncommon condition usually seen in patients with underlying conditions such as chronic alcoholism, malignancy, malnutrition, and hyponatremia [1]. We recently encountered a patient with AIDS and neurological abnormalities, whose evaluation was consistent with CPM.

A 38-year-old female patient with AIDS (most recent absolute CD4 cell count of 14 cells/μl) presented with a 4-week history of dysarthria, dysphagia, increasing generalized weakness, blurred vision, confusion, and difficulties with balance and walking. She was noncompliant with her prescribed medications (lopinavir/ritonavir 400 mg/100 mg twice daily, emtricitabine 200 mg daily, tenofovir 300 mg daily, atovaquone 1500 mg daily, and azithromycin 1200 mg weekly).

She was apyrexic with a blood pressure of 74/40 mmHg, a pulse of 102 beats/min, and a respiratory rate of 22 breaths/min. Oxygen saturation was 96% while breathing room air. There was no detectable lymphadenopathy or hepatosplenomegaly. Neurological examination was notable for horizontal nystagmus, dysarthria, ataxia, dysdiadokokinesia, and reduced muscle strength in the upper and lower extremities. Otherwise, the physical examination was unremarkable.

The results of a complete blood count, renal and hepatic function tests, and serum albumin and electrolyte values were normal as they had been on multiple occasions over the preceding several years. Magnetic resonance imaging (MRI) of the brain revealed diffuse edema in the pons, except for the dorsal aspect, consistent with CPM (Fig. 1). Cerebrospinal fluid (CSF) examination revealed one white blood cell and two red blood cells, glucose of 72 mg/dl, protein of 29 mg/dl, a nonreactive Venereal Disease Research Laboratory test, and a negative cryptococcal antigen assay. DNA amplification, by polymerase chain reaction, performed on the CSF was negative for toxoplasma, herpesviruses 1 and 2, cytomegalovirus, BK virus,

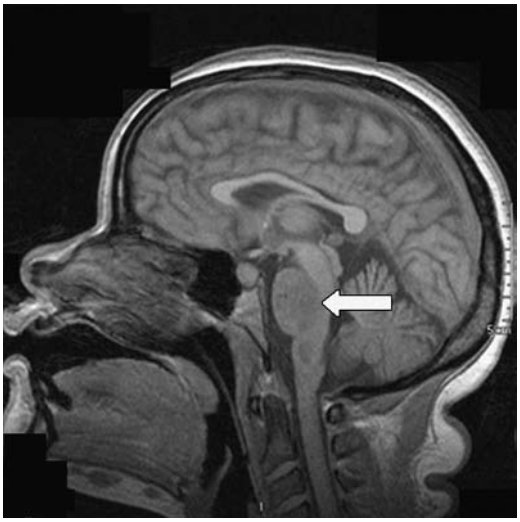

Fig. 1. Magnetic resonance imaging of the brain with a well defined lesion in the pons of low T_1-signal intensity (white arrow) consistent with central pontine myelinolysis.

and JC virus. CSF did not demonstrate oligoclonal bands suggestive of multiple sclerosis. *Histoplasma* antigen was not detected in the urine.

Empiric therapy for toxoplasmosis was begun, and her other medications were continued. She was discharged home but readmitted 1 month later with continued neurological decline. A repeat MRI of the head was again consistent with CPM, and other evaluation was unremarkable. Her neurological condition failed to improve, and she was ultimately transferred to a long-term care facility for terminal care. She continued with a decline in overall function and expired 3 months following her diagnosis with CPM. Permission for autopsy was not granted.

CPM is a demyelinization syndrome of the pons, which may be seen with rapid correction of hyponatremia [2] or other underlying conditions such as malnutrition, malignancy, alcoholism, burn injury, and chronic renal failure [1,3]. Severe neurological symptoms may result. Diagnosis is usually made at autopsy or on the basis of imaging studies.

The rare reports of CPM occurring in AIDS patients usually include other conditions that increase the potential for development of CPM [1,3–8]. The two patients described by Miller *et al.* [1] both had Kaposi sarcoma: one was found at autopsy to have lymphoma, and one had a history of heavy alcohol consumption. Holmes *et al.* [6] reported a case of CPM in an AIDS patient without preexistent hyponatremia [6]. Their patient, however, had prolonged hypoalbuminemia. By contrast, our patient was not known to have been hyponatremic or hypoalbuminemic or to have another identified condition known to be associated with CPM.

CPM in patients with HIV infection may not be recognized in life [1,5,6,8]. Clinical presentation may vary, ranging from mutism and dysarthria to spastic quadriparesis with a pseudobulbar palsy [2]. Lethargy and affective changes are common. Outcome is variable, ranging from complete recovery to death. Improvement may be gradual or partial with persisting bulbar dysfunction and spastic quadriparesis [2].

Our case illustrates that CPM should be considered as the cause for neurological disease in patients with AIDS in the appropriate clinical setting, regardless of serum sodium and albumin levels, especially if other diagnoses are excluded. If hyponatremia exists, caution should be exercised in its correction.

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Disruption of an env tyrosine-dependent sorting signal does not affect susceptibility of HIV-1 to cytotoxic T lymphocytes

Infections with live-attenuated viruses within SIV models of HIV-1 pathogenesis have offered the best evidence that a vaccine could generate protective immunity. Daniel *et al*. [1] first demonstrated this principle using *nef*-defective SIV, which established a chronic low-grade asymptomatic infection in macaques associated with protection against subsequent challenge with wild-type SIV. At least in part, this phenomenon appeared to be due to attenuation of viral replication.

More recently, Shacklett *et al*. [2] and Fultz *et al*. [3] have examined attenuated SIV infection through mutations in the transmembrane domain of gp41, which also subsequently protect from challenge by wild-type SIV. Shacklett *et al*. found that SIV containing multiple (stop and point) mutations disrupting this domain reduced viral replicative capacity *in vitro*, likely due to effects on gp41 membrane trafficking in infected cells [4,5]. Fultz *et al*. more specifically created mutations in the tyrosine-based sorting motif (Y712xxϕ) in the membrane-proximal cytoplasmic domain of gp41 [3]. Despite the role this motif in gp41 trafficking [4,5], the mutations appeared to have minimal impact on viral fitness, reflected by normal peak viremia during acute infection *in vivo* and growth kinetics *in vitro*. Thus, the mechanism of attenuation and subsequent immune protection is unclear, but probably not due to markedly reduced viral replication capacity. Interestingly, in-vivo CD8 depletion experiments have suggested that $CD8^+$ T lymphocytes (CTLs) may contribute to the protective immunity [6].

To assess whether analogous mutations in HIV-1 might affect viral susceptibility to CTLs, mutations in the Y712xxϕ motif were constructed in HIV-1 NL4-3 [7] by point mutagenesis (QuikChange, Stratagene). These included EnvY712I, EnvY712S and EnvΔGY mutations in the cytoplasmic domain of gp41 (Fig. 1a), engineered into the whole genome context of NL4-3.1 containing the clade B consensus sequence at Gag 77-85 (HXB2 amino acid numbering) [8]. These viruses were examined for their ability to replicate in T1 cells [9] (Fig. 1b). The EnvY712I and EnvY712S mutants had growth kinetics similar to those of wild-type NL4-3 (EnvY712), in turn similar to SIVmac239 [3]. In contrast, the EnvΔGY mutant was replication incompetent, suggesting that this mutation more severely impaired HIV-1 than SIV.

To assess the susceptibility of these mutants to CTLs, these viruses were tested in virus suppression assays [10,11] using HIV-1-specific CTL clones: S1-SL9-3.23T recognizing the HLA A*02-restricted epitope SLYNTVATL in Gag p17 (amino acid 77–85) [12], S31-IV9-10.11T recognizing the HLA A*02-restricted epitope ILKEPVHGV in reverse transcriptase (amino acid 309–317) and S58-PL10-10.8 recognizing the HLA A*02-restricted epitope PLTFGWCYKL in Nef (amino acid 136–145). The HIV-1-permissive target cells were A*02-expressing T1 cells [10]. Comparisons of these viruses showed similar degrees of suppression of the mutant and wild-type viruses by the three CTL clones (Fig. 1c and d). Similar results were noted in independent experiments using two other CTL clones recognizing other epitopes (not shown). Overall, these results suggested that directly increased susceptibility to CTL inhibition is not the mechanism of in-vivo attenuation of infection with viruses containing these mutations.

Because attenuated SIV infection has been the most robust example of protective vaccination in the SIV macaque model [1,6,13–15], understanding how viral attenuation affects antiviral immunity is clearly an important goal. The observation that SIV mutated in the Y712xxϕ motif yields infections with typical high peak viremia followed by chronic low viremia in macaques subsequently protected from wild-type virus challenge [2,3,6] suggests that viral replication is not markedly affected by the mutations, but that replication is suppressed during chronic infection after development of CTL responses, which are the major determinant of set-point viremia [16–18]. Furthermore, preliminary data suggest that the low set-point viremia of macaques infected with Y712xxϕ-disrupted SIV may be related to the CTL response [6]. Thus, a simple explanation could be that disruption of this motif somehow renders SIV directly more susceptible to CTL.

However, our results suggest that this is not the case for HIV-1; disruption of the Y712xxϕ motif did not appear to increase susceptibility of HIV-1 to CTLs directly. Although our in-vitro assay may not necessarily predict the interaction of virus and CTLs *in vivo*, it seems likely that the mechanism of attenuation is either not mediated by CTLs, or indirectly increases the antiviral activity of CTLs. Interestingly, it has been suggested that the HIV-1 Env may facilitate viral escape from CTLs in lymph nodes [19,20]. Although our data do not address this issue directly, they are compatible with a mechanism whereby reduced levels of Env could reduce viral escape from CTLs. Further work would be required to explore this possibility.

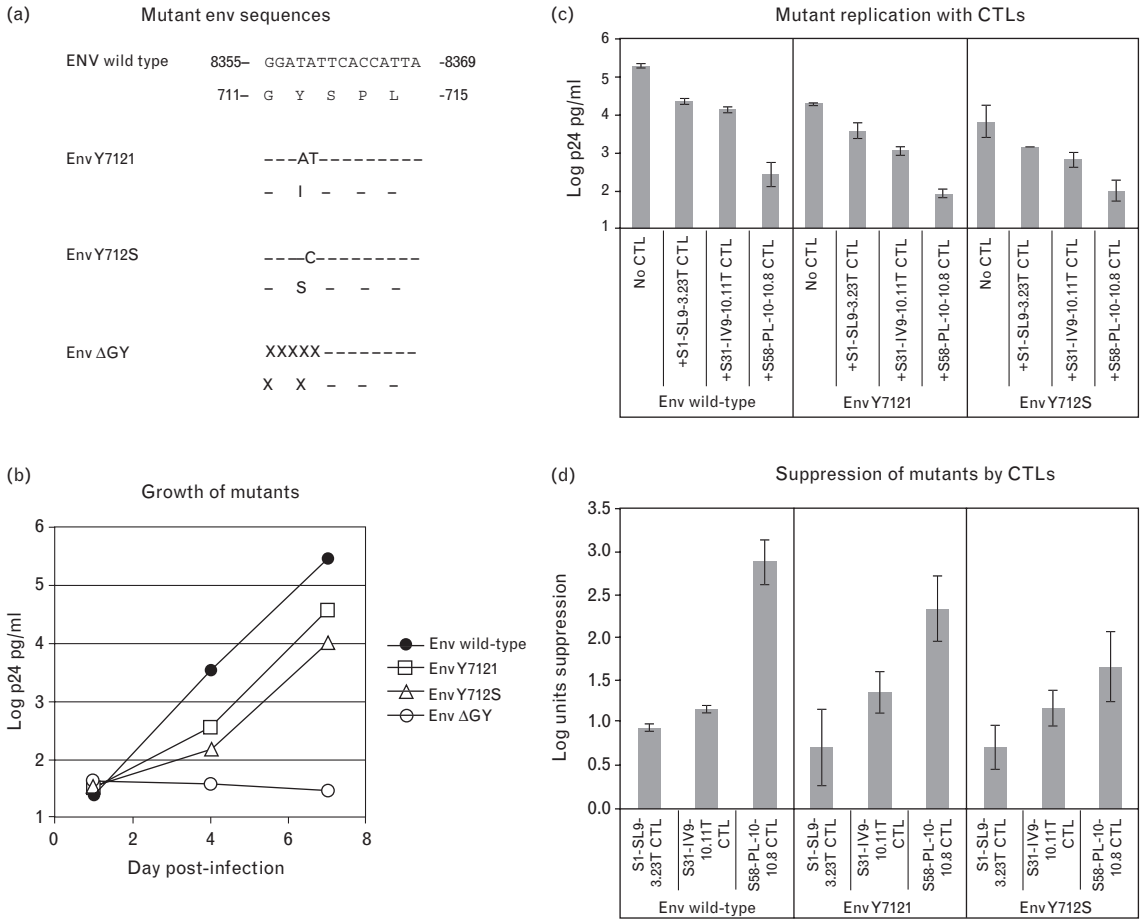

Fig. 1. Susceptibility of Env Y712xxϕ motif mutant viruses to HIV-1-specific CD8$^+$ T lymphocyte. (a) Nucleotide (NL4-3 residues 8355–8369) and amino acid (NL4-3 Env residues 711–715) sequences are given for the wild-type NL4-3.1 virus and generated mutants. Dash (–) indicates the same nucleotide or amino acid as wild type and cross (×) indicates a deletion of a nucleotide or amino acid compared with wild type. (b) 1×10^6 HIV-1 permissive T1 cells were infected with the indicated mutant or wild-type virus at an input of 1000 pg p24 antigen and cultured in a 24-well tissue culture plate. Viral replication was measured by quantitative p24 ELISA (Perkin–Elmer, Waltham, Massachusetts, USA). These results are representative of four independent experiments. (c) T1 cells were infected with 500 pg p24/10^6 cells and cultured in triplicate in 96-well tissue culture plates with the indicated CD8$^+$ T lymphocyte (CTL) clones (1.25×10^4 CTLs with 5×10^4 target cells). Viral replication was measured by quantitative p24 ELISA (Perkin–Elmer) on day 4 after infection. (d) Inhibition was calculated by comparing replication in cells with or without CTLs. The plotted data indicate means of triplicates (error bars indicate 1 SD) for one experiment, and the results are representative of two independent experiments with these clones. Similar results were seen using a B*57-restricted CTL clone recognizing the epitope TSTLQEQIGW in Gag p24 and an A*02-restricted CTL clone recognizing the epitope AIIRILQQL in Vpr in other experiments (data not shown).

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