Correspondence

AIDS 2007, 21:879–894

Treatment resistance after sequential interruption of a non-nucleoside reverse transcriptase inhibitor-based regimen

Stopping HIV therapy may reduce costs and side effects, but carries the risk of increased immune suppression and the emergence of resistance. To investigate this issue, the Strategies for Management of Anti-Retroviral Therapy (SMART) randomized study was conducted to compare the long-term consequences of two antiretroviral management strategies: continuous therapy versus scheduled treatment interruption. The study was interrupted for harm [1].

Non-nucleoside reverse transcriptase inhibitors (NNRTI), namely efavirenz and nevirapine, are frequently part of recommended combinations for the treatment of HIV-1 infection. NNRTI, however, have a low genetic barrier to the selection of resistance, and a single key mutation in the NNRTI-specific pocket site or in the surrounding domain of reverse transcriptase poses a major therapeutic problem for this class [2]. If administered as monotherapy, NNRTI selects for resistant mutants within one week, most commonly those harbouring the K103N or Y181C mutation, which confer cross-resistance to other approved drugs in the NNRTI class. The K103N mutation appears to have little effect on the replicative capacity of HIV-1, allowing variants to persist long after nevirapine therapy has been stopped. Resistance mutations have been observed after exposure to a single dose of nevirapine [3]. For the reasons described above, the SMART protocol recommended that NNRTI should be discontinued 7 days before nucleoside reverse transcriptase inhibitor (NRTI) backbone. Here we report the case of a patient who was included in the SMART study and who developed resistance after sequentially stopping a nevirapine-containing regimen.

Case report

A 44-year-old Caucasian man, diagnosed HIV-1 positive in 1994, was enrolled in SMART in March 2005 in the scheduled interruption treatment group. At this time, the CD4 lymphocyte count was 896 cells/μl (36%, CD4/CD8 0.87) and RNA HIV was undetectable (<50 copies/ml).

The nadir CD4 cell count was 2 cells/μl in 1995. He had no history of opportunistic infections. Lamivudine (300 mg once a day) was initiated in 1995. In April 1996, the patient received indinavir (2400 mg/day), lamivudine (300 mg/day) and didanosine (400 mg/day). In March 1998, indinavir was stopped and switched to ritonavir (1000 mg/day). NNRTI with efavirenz (600 mg/day) substituted ritonavir in May 1999 for simplification. Efavirenz was switched to nevirapine (400 mg/day) in August 2003. Our case had had undetectable RNA HIV of less than 400 copies/ml since 1996 and less than 50 copies/ml since 1999 in all 3-month routine follow-ups until May 2005. According to the protocol, he stopped nevirapine on 12 April 2005 and stopped didanosine and lamivudine on 19 April 2005. His CD4 lymphocyte count decreased (Table 1).

In October 2005, the CD4 lymphocyte count was 129 cells/μl and according to the SMART protocol, the treatment was reinitiated with cotrimoxazole for the prevention of opportunistic infections. We chose to restart the same treatment with didanosine (400 mg), lamivudine (300 mg) and nevirapine (200 mg once a day for 14 days and then 200 mg twice a day). An HIV-1 genotypic resistance test for each drug was performed in March 2006, and revealed Y181C and M184I reverse transcriptase mutations, which confer drug resistance to NNRTI and lamivudine, respectively. The NRTI backbone was changed and lopinavir–ritonavir replaced nevirapine with virological control.

NNRTI is an attractive class of treatment for HIV-1 infection because of its good bioavailability, long half-life

Table 1. Immunovirological history according to antiretroviral therapy.

<table>
<thead>
<tr>
<th>May</th>
<th>August</th>
<th>March</th>
<th>May</th>
<th>June</th>
<th>July</th>
<th>August</th>
<th>October</th>
<th>January</th>
<th>March</th>
<th>May</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD4 cell count (cells/μl)</td>
<td>767</td>
<td>836</td>
<td>1018</td>
<td>294</td>
<td>223</td>
<td>296</td>
<td>245</td>
<td>129</td>
<td>179</td>
<td>106</td>
</tr>
<tr>
<td>RNA-HIV viral load Copies/ml</td>
<td>&lt; 50</td>
<td>&lt; 50</td>
<td>&lt; 50</td>
<td>86 524</td>
<td>14063</td>
<td>7220</td>
<td>18,585</td>
<td>31,103</td>
<td>5379</td>
<td>7894</td>
</tr>
<tr>
<td>Treatment</td>
<td>3TC</td>
<td>3TC</td>
<td>3TC</td>
<td>Stopped</td>
<td>Stopped</td>
<td>Stopped</td>
<td>Stopped</td>
<td>Stopped</td>
<td>Stopped</td>
<td>Stopped</td>
</tr>
<tr>
<td></td>
<td>ddI</td>
<td>ddI</td>
<td>ddI</td>
<td>3TC</td>
<td>3TC</td>
<td>3TC</td>
<td>3TC</td>
<td>ddI</td>
<td>ddI</td>
<td>ddI</td>
</tr>
<tr>
<td>EFV</td>
<td>NVP</td>
<td>NVP</td>
<td>NVP</td>
<td>NVP</td>
<td>NVP</td>
<td>NVP</td>
<td>NVP</td>
<td>NVP</td>
<td>NVP</td>
<td>NVP</td>
</tr>
</tbody>
</table>

ddI, Didanosine; EFV, efavirenz; NVP, nevirapine; 3TC, lamivudine.

*dNevirapine stopped 7 days before didanosine and lamivudine.
and convenient administration. It should be stressed that the necessity to stop NNRTI is not uncommon in clinical practice, because of adverse events, concomitant conditions or other patient non-medical related factors. Two factors have been associated with the emergence of NNRTI resistance: previous suboptimal treatment [4] and treatment interruptions [5,6]. Both were present in our case, and the emergence of resistance occurred despite sequential treatment interruption. Even with relatively long-term virological control, antiretroviral therapy cessation clearly triggered resistance in this case.

Our report has potential important implications when stopping an NNRTI, in particular in patients with previous NRTI suboptimal treatment. The current protocol for NNRTI cessation requires that two NRTI alone are sufficient to control RNA-HIV replication during the 7 days of NNRTI clearance, and thus avoid HIV replication in the presence of subtherapeutic NNRTI levels [7]. This may not be possible in patients with previous suboptimal NRTI treatment, suggesting the presence of archived resistant viruses, even in the case of long-term virological control. We suggest that in this setting, protease inhibitors should replace NNRTI in addition to NRTI, during the delay of NNRTI clearance (which remains to be determined) in order to preserve this potent antiretroviral option.

Acknowledgement

We wish to dedicate our work to our SMART volunteer who consented to make a sacrifice of himself for the good of others. The authors also thank Pascale Goubin for data management.

Effectiveness and durability of non-nucleoside reverse transcriptase inhibitor-based therapy in HIV clinical practice

In a recent review of triple combination therapy trials among treatment-naive HIV patients updated from their earlier meta-analysis, Bartlett and colleagues [1,2] found superior viral suppression in antiretroviral therapy (ART) regimens containing non-nucleoside reverse transcriptase inhibitors (NNRTI) and boosted protease inhibitors (PI). Convenient dosing, lower pill burdens, better tolerance, and PI-sparing have made NNRTI-based regimens pivotal in ART initiation. NNRTI efficacy for treatment-naive HIV patients has been assessed during clinical trials [1–4] lasting 24 weeks for drug approval from the US Food and Drug Administration, and data on fewer patients remaining on their treatment arms are available to 96 weeks. Lipid profile differences have been shown for patients treated with NNRTI compared with PI [5]. Little is known about the long-term clinical outcomes despite reports of NNRTI use in clinical practice [6,7]. We assessed not only the effectiveness of NNRTI-based initial ART in clinical practice, but also the development of coronary artery disease, diabetes mellitus and hyperlipidemia in treatment-naive HIV patients.

A retrospective review of treatment-naive patients assessed changes in the CD4 cell count, HIV-RNA level, lipid profiles, the development of HIV genotypic mutations and co-morbidities such as coronary artery disease, diabetes mellitus, and hyperlipidemia during NNRTI-based initial ART without PI during 1998–2004. Hypertension served as a control prevalent co-morbidity. Continuous and categorical variables were analysed by Student's t-test and by Fisher’s exact test, respectively.

References


Forty-nine patients without the four exclusionary co-morbidities started NNRTI-based ART. Their median age was 44 years (range 30–64), 98% were men, and 82% were African-American. The total follow-up for all patients was 1420 patient-months. Nineteen patients had pre-AKT clinical events or opportunistic infections. All patients initiated ART with two or more nucleoside/nucleotide reverse transcriptase inhibitors (NRTI; such as didanosine, lamivudine, stavudine, tenofovir, and zidovudine) and a single NNRTI (40 efavirenz and nine nevirapine). Two individuals changed from efavirenz to nevirapine because of disturbing dreams and depression on efavirenz. None received concomitant didanosine and tenofovir.

Table 1 summarizes laboratory data before and on therapy. Among 21 patients with baseline CD4 cell counts of less than 200 cells/μl, 76% achieved CD4 cell counts greater than 200 cells/μl on NNRTI. CD4 cell increases were sustained, and no patient developed any adverse events of immune reconstitution. Complete viral suppression was achieved in 87% of patients tested at a median of 125.5 days after baseline. These CD4 cell counts and viral responses exceeded earlier reports [1–4, 6, 7]. Among 40 patients with undetectable viremia, 75% sustained complete viral suppression for a median of 728 days (range 28–2071), and 25% failed with circulating median HIV RNA of 2825 copies/ml (range 82–53,698) after median treatment of 23 months (range 6–49). K103N was the only mutation detected in three patients. As NNRTI have a low genetic resistance barrier [8], our results contrast with an earlier report in which NNRTI-based ART was shown for the total cholesterol : HDL ratio (0.7 with 95% confidence interval 0.2–1.3), and the lipid changes in Table 1 suggest a less atherogenic profile on NNRTI therapy [5]. A reversal of these lipid changes was observed before or at the time of detectable viremia for a few patients with virological failure, implying that those patients were not fully adherent to ART, or viral replication may have a direct effect on lipid profiles [11]. In contrast to published reports [11–13], no lipid differences were observed between nevirapine compared with efavirenz treatment. For clinical outcomes, two patients (4%) developed hyperlipidemia 8 and 11 months after starting NNRTI-based ART. No patients had documented diabetes mellitus or coronary artery disease on NNRTI. One patient (2%) developed hypertension (control morbidity) 19 months after starting nevirapine-based ART. No patients had documented diabetes or coronary artery disease. All patients compared with hypertension (control) in 2%; none developed diabetes or coronary artery disease. All patients survived with no clinical progression.

Table 1. Comparison of laboratory parameters before and during non-nucleoside reverse transcriptase inhibitor-based therapy.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Before NNRTI (μ)</th>
<th>During NNRTI (μ)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD4 cell count (cells/μl)</td>
<td>205±149 (48)</td>
<td>311±205 (47)</td>
<td>0.0048*</td>
</tr>
<tr>
<td>CD4 cell percentage</td>
<td>12±7 (48)</td>
<td>18±9 (47)</td>
<td>0.0005*</td>
</tr>
<tr>
<td>HIV RNA (copies/ml)</td>
<td>96,423±128,770 (48)</td>
<td>1671±10,076 (46)</td>
<td>&lt;0.0001*</td>
</tr>
<tr>
<td>Log_{10} HIV RNA</td>
<td>4.65±0.58 (48)</td>
<td>2.04±0.53 (46)</td>
<td>&lt;0.0001*</td>
</tr>
<tr>
<td>Undetectable HIV RNA</td>
<td>0 (48)</td>
<td>40 (46)</td>
<td>&lt;0.0001*</td>
</tr>
<tr>
<td>Cholesterol (mg/dl)</td>
<td>171±41 (33)</td>
<td>186±43 (40)</td>
<td>0.1279*</td>
</tr>
<tr>
<td>HDL (mg/dl)</td>
<td>41±13 (31)</td>
<td>51±15 (39)</td>
<td>0.0045*</td>
</tr>
<tr>
<td>LDL (mg/dl)</td>
<td>90±39 (13)</td>
<td>112±36 (29)</td>
<td>0.0792*</td>
</tr>
<tr>
<td>LDL : HDL</td>
<td>2.3±1.0 (12)</td>
<td>2.1±0.9 (30)</td>
<td>0.3607*</td>
</tr>
<tr>
<td>Cholesterol : HDL</td>
<td>4.3±1.1 (31)</td>
<td>3.6±1.1 (37)</td>
<td>0.0093*</td>
</tr>
<tr>
<td>Triglycerides (mg/dl)</td>
<td>159±105 (14)</td>
<td>134±98 (31)</td>
<td>0.4459*</td>
</tr>
</tbody>
</table>

HDL, High-density lipoprotein; LDL, low-density lipoprotein; NNRTI, non-nucleoside reverse transcriptase inhibitor.

*aUnpaired two-tailed t-test.

*bFor viral load outside the detectable range, 74 and 500,001 copies were used for calculations.

*cTwo-tailed Fisher’s exact test.

More patients achieved high-density lipoprotein (HDL) of 40 mg/dl or greater compared with baseline (31/39 versus 15/31, P=0.0107). A difference between baseline and NNRTI treatment was shown for the total cholesterol : HDL ratio (0.7 with 95% confidence interval 0.2–1.3), and the lipid changes in Table 1 suggest a less atherogenic profile on NNRTI therapy [5]. A reversal of these lipid changes was observed before or at the time of detectable viremia for a few patients with virological failure, implying that those patients were not fully adherent to ART, or viral replication may have a direct effect on lipid profiles [11]. In contrast to published reports [11–13], no lipid differences were observed between nevirapine compared with efavirenz treatment. For clinical outcomes, two patients (4%) developed hyperlipidemia 8 and 11 months after starting NNRTI-based ART. No patients had documented diabetes mellitus or coronary artery disease on NNRTI. One patient (2%) developed hypertension (control morbidity) 19 months after starting nevirapine-based ART. No AIDS-defining events or deaths occurred. In clinical practice, NNRTI-based initial ART was durable and effective for CD4 cell response, viral suppression, and lipid profile improvement despite suboptimal adherence. Hyperlipidemia developed in 4% of patients compared with hypertension (control) in 2%; none developed diabetes or coronary artery disease. All patients survived with no clinical progression.
Immune reconstitution syndrome in tuberculosis and HIV-co-infected patients: Th1 explosion or cytokine storm?

The article ‘Explosion of tuberculin-specific Th1 responses induces immune restoration syndrome in tuberculosis and HIV co-infected patients’ by Bourgarit and colleagues [1] explores the immunological changes underlying the immune reconstitution syndrome (IRS). The paper is the first to apply a broad proteomic approach, and highlights very important aspects of this complex immunological phenomenon.

The authors analyse the in-vitro production of 25 inflammatory and immunomodulatory cytokines, chemokines and soluble cytokine receptors in supernatants of purified protein derivative-stimulated peripheral blood mononuclear cells from four patients (three undergoing IRS, one not undergoing IRS). The authors classify the immune molecules in the following fashion: T helper cell (Th) type 1-related cytokines/chemokines (IFN-γ, IL-2, IL-12, IFN-γ-inducible protein 10 and monokine induced by IFN-γ); Th2 cytokines (IL-4, IL-5, IL-13 and IL-15); inflammatory cytokines/chemokines (TNF-α, IL-6, IL-1b, IL-10, regulated upon activation: normal T cell expressed/secreted and monocyte chemoattractant protein 1). The markers IL-1 receptor antagonist (IL-1RA), IL-2 receptor, IL-7, IL-8, IL-17, granulocyte macrophage colony-stimulating factor, macrophage inflammatory proteins 1α and 1β are measured, but data are not presented. On the basis of this classification of cytokines, the authors propose ‘that an excessive restoration of purified protein derivative-specific Th1 response with no Th2 balance is responsible for the enlargement of TB granuloma lesions and is associated with an acute release of non-specific pro-inflammatory cytokines and chemokines inducing the systemic inflammatory syndrome’. In the present study, however, IL-10 is classified as a non-specific inflammatory cytokine, and IL-15 as a Th2 cytokine. IL-10 is well established as a classic Th2 and anti-inflammatory cytokine [2]. IL-15 is a pro-inflammatory cytokine sharing many functions with IL-2 [3]. With the correct classification of the presented cytokine measurements, the results are an increase in purified protein derivative-specific Th1 cytokines/chemokines (IFN-γ, IL-2, IL-12, IFN-γ-inducible protein 10 and monokine induced by IFN-γ), in non-specific inflammatory cytokines/chemokines (TNF-α, IL-6, IL-1β), regulated upon activation: normal T cell expressed/secreted and monocyte chemoattractant protein 1), and in the Th2/anti-inflammatory cytokine IL-10, coinciding with the development of IRS symptoms.
loss of thymic-derived cell populations (naive T cells [8]) could be the consequence of the fact that an HIV-induced immunopathogenesis [6], which drives a deregulated immune activation [7] and expansion of the two non-specific regulatory cytokines IL-10 and TGF-β. Although measured, the authors fail to comment on the cytokine kinetics into consideration [5].

On the basis of current knowledge it is tempting to hypothesize that the immunological basis of IRS is a HAART-induced rapid clonal expansion and redistribution of Mycobacterium tuberculosis-specific memory T cells [6], which drives a deregulated immune activation [7] and a cytokine storm [1]. The deregulated immune function could be the consequence of the fact that an HIV-induced loss of thymic-derived cell populations (naive T cells [8] and natural regulatory T cells [9,10]) are restored at much slower kinetics compared with the peripheral memory T-cell population after HAART [6,8]. Tuberculosis-specific regulatory T cells have recently been demonstrated [11], and murine data have shown that regulatory T cells dampen the symptoms of IRS [12]. Therefore, because of a lack of naturally occurring regulatory T cells in the first months of HAART treatment, there is a basis of deregulated memory T-cell expansion leading to a cytokine storm and the development of IRS.

**Fig. 1. Antigen-specific IL-1 receptor antagonist production in whole blood culture.** Whole blood of eight Quantiferon In Tube-positive, culture or sputum-positive tuberculosis (TB) patients and seven healthy tuberculosis-unexposed controls was stimulated for 20–24 h with either saline or Mycobacterium tuberculosis-specific antigens in the Quantiferon In Tube system. IL-1 receptor antagonist (IL-1RA) production was measured in 1:8 diluted plasma by multiplex technology on the Luminex platform (Luminex, Riverside, California, USA) using Biosource reagents (Biosource, Camarillo, California, USA). Antigen-specific production represent the antigen-stimulated sample subtracted from the saline sample. Straight lines represent median values. Differences between healthy controls and patients were significant at \( P < 0.0006 \) (Kruskal–Wallis test).

Therefore, on the basis of the data presented in the article, the IRS seems more likely to be induced by an explosion of Th1, Th2, and non-specific inflammatory mediators simultaneously, i.e. a cytokine storm [4].

Although measured, the authors fail to comment on the production of the two non-specific anti-inflammatory immune effectors IL-1RA and soluble IL-2 receptor. As non-specific anti-inflammatory cytokine and soluble receptor production represent naturally occurring inflammation inhibitors, the study of markers from this class is essential for the understanding of IRS. We have recently found high levels of IL-1RA in 1:8 diluted plasma of whole blood culture stimulated with the tuberculosis specific antigens ESAT-6, CFP-10 and TB7.7 (unpublished observations). Patients with active tuberculosis produced antigen-specific IL-1RA responses of a median 2282 pg/ml (range 162–4432 pg/ml; Fig. 1), equivalent to 18 256 pg/ml in undiluted sample. Future studies are needed to elucidate the effects of these immune mediators during IRS, and should compare whole blood with peripheral blood mononuclear cell culture and take cytokine kinetics into consideration [5].

On the basis of current knowledge it is tempting to hypothesize that the immunological basis of IRS is a HAART-induced rapid clonal expansion and redistribution of Mycobacterium tuberculosis-specific memory T cells [6], which drives a deregulated immune activation [7] and a cytokine storm [1]. The deregulated immune function could be the consequence of the fact that an HIV-induced loss of thymic-derived cell populations (naive T cells [8] and natural regulatory T cells [9,10]) are restored at much slower kinetics compared with the peripheral memory T-cell population after HAART [6,8]. Tuberculosis-specific regulatory T cells have recently been demonstrated [11], and murine data have shown that regulatory T cells dampen the symptoms of IRS [12]. Therefore, because of a lack of naturally occurring regulatory T cells in the first months of HAART treatment, there is a basis of deregulated memory T-cell expansion leading to a cytokine storm and the development of IRS.

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**References**


Atazanavir in plasma-exchange treatment

Atazanavir is an HIV protease inhibitor (PI) with a pharmacokinetic profile that allows once daily oral administration. As with other PIs, atazanavir is primarily metabolized by CYP3A isoenzymes in the liver. Therefore, the drug is prone to drug–drug interactions. The terminal elimination half-life of atazanavir administered as a single agent is 6–7 h. Atazanavir is 86% bound to human serum protein. The recommended atazanavir doses are 400 mg once a day taken with food or 300 mg once a day when boosted with low-dose ritonavir (100 mg) [1].

Because atazanavir is a relatively new agent, published pharmacokinetic data on this compound are scarce thus far. No data are available on patients receiving plasma-exchange treatment. Plasma exchange is a procedure in which plasma is separated from blood cells, removed and exchanged for donor plasma before returning it to the patient. Plasma exchange can reduce the amount of abnormal protein in the blood, but may also significantly reduce levels of drugs in the blood (both protein-bound and unbound drug levels).

We report a patient receiving antiretroviral medication including atazanavir and plasma-exchange treatment. In this patient we monitored atazanavir plasma concentrations before and after plasma-exchange treatment in order to study the influence of plasma exchange on the atazanavir pharmacokinetics. Atazanavir was assayed using a liquid chromatography–mass spectrometry assay with a lower limit of quantitation of 0.1 mg/l, as used in our hospital [2].

A 41-year-old man was admitted to our hospital with a diagnosis of thrombotic thrombocytopenic purpura (TTP), possibly related to an HIV infection diagnosed at the same time (HIV viral load 9.5 × 10^5 copies/ml, CD4 cells 190 × 10^6/l). The patient had an adequate liver test and slightly decreased renal function (creatinine 112 μmol/l, alanine aminotransferase 20 IU/l, aspartate aminotransferase 40 IU/l, gammaglutamyl transferase 21 IU/l). The patient started with antiretroviral treatment including atazanavir (300 mg once a day), ritonavir (100 mg once a day), tenofovir (245 mg once a day) and emtricitabine (200 mg once a day). On the same day, the patient received phenytoin for epileptic seizures, which were neurological complications of TTP. Plasma exchange was performed by a continuous centrifugation technique. The amount of plasma removed per plasma-exchange session was 3000 ml, corresponding to 40 ml/kg of the patient’s body weight. Volume replacement fluid was fresh frozen donor plasma. The amount substituted was equal to that of the removed plasma. Every day, the process of plasma exchange took approximately 2 h. From day 10 of treatment, blood samples were withdrawn for atazanavir monitoring just before and directly after three plasma-exchange sessions. The time between the two blood samples was approximately 3 h. Antiretroviral medication was given after the post-plasma-exchange sample was drawn, making the pre-plasma-exchange sample a ‘trough’ sample. At days 9, 11, 14, 15 and 16, no plasma-exchange treatment took place. The last plasma-exchange session was on day 17. That same day, atazanavir treatment was discontinued and replaced by efavirenz.

From 2 days before the start of antiretroviral therapy, the patient received phenytoin for epileptic seizures, which were neurological complications of TTP (150 mg twice a day during the first 5 days; 75 mg twice a day during the following 4 days; 75 mg once a day on subsequent days). Phenytoin is known to be a potent inducer of CYP3A4 and may therefore enhance atazanavir metabolism.

In Table 1 the results of the atazanavir plasma concentration measurements are shown. For comparison, phenytoin concentrations in the same samples are also reported. The results show that atazanavir plasma concentrations were reduced by one half during the 2-h process of plasma-exchange treatment as applied in this patient. As atazanavir half-life when administered in

<table>
<thead>
<tr>
<th>Day of atazanavir treatment</th>
<th>Concentration of atazanavir before PE session (mg/l)</th>
<th>Concentration of atazanavir after PE session (mg/l)</th>
<th>Concentration of phenytoin before PE session (mg/l)</th>
<th>Concentration of phenytoin after PE session (mg/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>3.5</td>
<td>1.5</td>
<td>2.75</td>
<td>2.42</td>
</tr>
<tr>
<td>13</td>
<td>4.8</td>
<td>2.6</td>
<td>0.9</td>
<td>0.84</td>
</tr>
<tr>
<td>17</td>
<td>2.7</td>
<td>1.5</td>
<td>0.39</td>
<td>0.37</td>
</tr>
</tbody>
</table>

PE, Plasma exchange.
combination with ritonavir and tenofovir has been reported to be 8–10 h [3], plasma-exchange treatment appears to reduce the half-life of atazanavir approximately fourfold. This significant effect of plasma exchange on atazanavir concentrations was expected as a result of the low distribution of atazanavir in body fluids and a number of tissue compartments [1]. Therefore, the majority of atazanavir in the body will be present intravascularly, and will be removed and diluted with plasma-exchange treatment. In contrast, the volume of distribution of phenytoin is relatively large (0.5–1.0 l/kg). The effect of plasma-exchange treatment on phenytoin plasma concentrations is therefore expected to be minor, because replacement of the intravascular fluid will have a minor effect on the total amount of phenytoin in the body.

In conclusion, plasma-exchange treatment has a significant effect on atazanavir clearance. As concentration–effect relationships for atazanavir have been described [4], we suggest that when treating a patient using atazanavir plasma-exchange treatment has a significant effect on atazanavir clearance. Atazanavir administration should take place directly after a plasma-exchange session.

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References


Partial splenic embolization as a treatment for hypersplenism in HIV/hepatitis C virus-co-infected patients

The development of thrombocytopenia in the course of untreated chronic hepatitis C may be caused by autoimmune phenomena or the infection of platelets with hepatitis C virus (HCV), but it generally denotes portal hypertension or, in other words, advanced hepatic fibrosis or cirrhosis [1], so it carries a bad prognosis. The only therapeutic option for patients with chronic hepatitis C at present is a regimen of interferon plus ribavirin, and as the dose of both interferon and ribavirin is crucial for an optimal response [2], the haematological toxicity of this regimen must be managed with the addition of haemopoietic factors rather than by reducing the drug doses [3,4]. Experience with recombinant thrombopoietin in patients with low platelet counts is scarce, and its efficacy seems to be low [3], therefore the development of thrombocytopenia with less than 50 000 platelets/μl is a frequent cause of treatment withdrawal. On the other hand, advanced liver disease is associated with a more severe thrombocytopenia, which precludes optimal treatment in the group of patients that more critically need it. Apart from splenectomy and splenic irradiation, there are no effective therapies for this kind of thrombocytopenia. Splenic irradiation has not been proved to increase platelet counts in patients with HIV-related thrombocytopenia, and has a negative impact on immunological status [5]. Splenectomy brings about a considerable morbimortality, particularly in patients with portal hypertension, and carries an increased risk of immediate and delayed infectious complications [6]. There have been good results with laparoscopic splenectomy, but it renders patients asplenic, and data on safety in HIV/HCV-co-infected patients are lacking. Partial splenic embolization (PSE) has proved to be a useful method to resolve thrombocytopenia caused by hypersplenism [7,8]. Here we report our experience on PSE in eight HIV/HCV-co-infected patients unable to tolerate an interferon/ribavirin regimen because of thrombocytopenia secondary to hypersplenism (Table 1).

All the patients, formerly intravenous drug users, had HCV-associated liver disease, and at the time of PSE all had cirrhosis; one was in Child–Pugh class B and the remainder were in class A. Hypersplenism was diagnosed by the observation of an ultrasonographically enlarged spleen (range 480–4224 ml) and reduced blood platelet counts (range 53–134 × 10\textsuperscript{10}/μl). All were on successful antiretroviral therapy, with undetectable plasma HIV viral loads and CD4 cell counts of 74–667 cells/μl (median 407). Pneumococcal, Haemophilus influenzae and quadrivalent meningococcal vaccines were administered, and, after written consent, a catheter was placed, via the femoral artery, in the splenic artery distal to the dorsal pancreatic branch. Embolization was performed injecting polyvinyl particles of approximately 300–700 μm.
antibiotics were also injected and continuous angiographic control was performed, so the procedure could be stopped when approximately 70% of the splenic parenchyma was embolized. All patients received intravenous analgesic (meperidine) and antiemetic (metoclopramide) medication and antibiotic prophylaxis during and after the procedure. Complications were remarkably absent, except in one patient in whom the procedure was very difficult and resulted in a massive splenic infarct, sterile peritonitis presumably caused by the injected material and later secondary bacterial peritonitis. Another patient was readmitted for persistent fever, which was proved to be secondary to a subtotal splenic infarction; in addition, he was diagnosed as having an asymptomatic splenic and left portal branch vein thrombosis. Pain was always present and required narcotic analgesics in most cases; fever was also common, but it was caused by an infectious complication in only one case. A normal platelet count was achieved in every case, and all except two patients have been able to tolerate the subsequent regimen of full-dose pegylated interferon and ribavirin; in one patient thrombocytopenia relapsed (later, several accessory spleens were discovered at laparoscopy), and another developed neutropenia refractory to granulocyte colony-stimulating factor during such regimen (another patient stopped treatment against medical advice). Five out of eight patients completed a 48-week full-dose regimen; of these, three are sustained viral responders, one is an end-of-treatment responder and one, who was an end-of-treatment responder, died later from a septic shock.

PSE preserves some residual splenic function and can restore a normal platelet count without major complications. It has recently been recommended as a pretherapy in HCV-infected patients with hypersplenism [7], including liver transplant patients [8]. Foruny et al. [9] reported their experience with eight patients, of whom three were HIV/HCV co-infected, and in all cases thrombocytopenia corrected after PSE, allowing the completion of a full-dose anti-HCV regimen in 75% of patients. In that series asymptomatic portal thrombosis developed in four patients, compared with one patient from our series.

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Table 1. Clinical features, tolerance to partial splenic embolization and hepatitis C virus treatment status of the patients.

<table>
<thead>
<tr>
<th>No.</th>
<th>Age</th>
<th>HCV viral load</th>
<th>Platelets pre-PSE</th>
<th>SI</th>
<th>Splenic index</th>
<th>Fever</th>
<th>HD</th>
<th>Opiates</th>
<th>Complications</th>
<th>Treatment status</th>
<th>Infections post-PSE</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>48</td>
<td>&gt;850000/3a</td>
<td>964000</td>
<td>124</td>
<td>422</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
<td>Treatment with ETVR</td>
<td>No</td>
</tr>
<tr>
<td>2</td>
<td>39</td>
<td>&gt;850000/3a</td>
<td>834000</td>
<td>120</td>
<td>143</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
<td>Treatment with SVR</td>
<td>No</td>
</tr>
<tr>
<td>3</td>
<td>44</td>
<td>&gt;850000/3a</td>
<td>450000</td>
<td>120</td>
<td>45</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
<td>Treatment with SVR</td>
<td>No</td>
</tr>
<tr>
<td>4</td>
<td>40</td>
<td>432000/3a</td>
<td>800000</td>
<td>120</td>
<td>154</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
<td>Treatment with SVR</td>
<td>No</td>
</tr>
<tr>
<td>5</td>
<td>40</td>
<td>100000/3a</td>
<td>480000</td>
<td>120</td>
<td>134</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
<td>Treatment with SVR</td>
<td>No</td>
</tr>
<tr>
<td>6</td>
<td>37</td>
<td>850000/3a</td>
<td>810000</td>
<td>120</td>
<td>157</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
<td>Treatment with SVR</td>
<td>No</td>
</tr>
<tr>
<td>7</td>
<td>46</td>
<td>&gt;850000/3a</td>
<td>100000/3a</td>
<td>120</td>
<td>157</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
<td>Treatment with SVR</td>
<td>No</td>
</tr>
<tr>
<td>8</td>
<td>41</td>
<td>&gt;850000/3a</td>
<td>500000</td>
<td>120</td>
<td>156</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
<td>Treatment with SVR</td>
<td>No</td>
</tr>
</tbody>
</table>

EVR, Early virological response; ETVR, end-of-treatment virological response; HD, hospitalization days; PSE, partial splenic embolization; SI, splenic index; or the product of the multiplication of the three splenic diameters in ml (normal until 440); SS, septic shock; SVR, sustained virological response.

References
Response to Morrison et al., ‘Hormonal contraception and the risk of HIV acquisition’

The results reported by Morrison and colleagues [1] provide encouragement that hormonal contraceptives may not affect a woman’s risk of HIV acquisition. As the authors note, however, their observational study design had limitations that compromise full confidence in their conclusions. Because of the vital importance of the issue, especially in light of the increasing popularity of hormonal methods in areas of high HIV incidence, more robust data are desirable. The accompanying editorial by Bulterys et al. [2] asserts that ‘unfortunately, no randomized clinical trial can be conducted owing to ethical and adherence issues resulting from randomly assigning women to contraceptive methods with substantially differing efficacy.’ We agree, but a randomized study that compares methods with similar efficacy should be possible. To investigate the association between hormonal methods and HIV infection, women at risk could be assigned to use either a long-acting hormonal contraceptive, such as an injectable or an implant, or a copper-containing intrauterine device, which has no known effect on HIV infection [3]. These methods have similar high contraceptive efficacy and minimal adherence problems, and current data and guidelines allow their safe use in women at risk of HIV exposure if appropriate screening is performed at initiation [4]. This design would eliminate the very serious concern of selection bias. Although such a trial could not easily be masked, differences in relevant behavior between groups after enrollment (such as condom use) and in the validity of reporting of these behaviors would probably be less than in the study by Morrison et al. [1]. We [5] and others (E. Stringer, personal communication) have recently completed successful studies confirming the feasibility of the randomized design, and we are currently conducting additional preparatory investigations in anticipation of a larger trial.

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References


Child sexual abuse, HIV, and tuberculosis in sub-Saharan Africa: a case report from rural Cameroon

Most people affected by HIV and tuberculosis live in sub-Saharan Africa [1], where young adults have the highest incidence of both diseases [2,3]. HIV transmission routes are well established in most adults and young children, but are sometimes unclear in older children and teenagers [4]. Sexual abuse may play an important role in this age group.

A 12-year-old boy presented to a hospital in rural northwest Cameroon with a one-year history of...
productive cough and weight loss despite multiple antibiotics. On consultation, there were night sweats and decreased appetite, no rigors or haemoptysis, respiratory rate was 28 breaths per minute, blood pressure was 80/60 mmHg, temperature was 36.6°C, and pulse was 100 beats per minute. He appeared chronically ill, with submandibular lymphadenopathy, bilateral coarse breath sounds, and mild clubbing.

In Cameroon, HIV is frequently associated with CSA. In Douala, 24 out of 71 sexually abused children (mean age 11.6 years) tested for HIV became seropositive as a result of penetration [13]. Abuse involved rape 84% of the time and mostly occurred outside the family. A national policy to protect children, incorporating post-exposure prophylaxis, was recommended. In Yaounde schools, 269 out of 1710 students reported sexual abuse before the age of 16 years [14]. CSA occurred at school 15% of the time and involved classmates one third of the time, although teachers and tutors were also implicated.

This case illustrates the problem of CSA in sub-Saharan Africa and suggests the transmission of HIV as a result of penetrative sex by an older male relative. Caretakers of CSA patients often refuse to report assault to police because of fear of social stigma and possible hostility from authorities [15]. An increased understanding of these phenomena is crucial in countries with high HIV prevalence, as the epidemic may be increasing children’s risk of rape and incest [10]. HIV post-exposure prophylaxis after CSA was found to be acceptable, safe, and feasible in a resource-poor setting [16] and is increasingly included in national guidelines. Abuse that occurs within children’s communities requires at least as much attention as the commercial sexual exploitation of children targeted by international organizations [17]. Education and public awareness about CSA, stigma reduction, and the enforcement of relevant laws must become priorities in Africa to help reduce the burden of HIV.

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References


Loss of HIV-1-specific T-cell responses associated with very rapid HIV-1 disease progression

The development of AIDS during the first year after acute infection is rare (less than 0.5% of cases) [1–5], and although cases of rapid progression to AIDS have been reported [4], the underlying factors are not well understood. Here we describe the case of an HIV-1-infected man with rapid disease progression, reaching a CD4 cell count of less than 50 cells/µl within 14 months of initial infection. An in-depth analysis of the evolution of virological and immunological characteristics in this patient identified a very weak to absent neutralizing antibody response and a significant loss of detectable HIV-1-specific CD8 T-cell responses in the absence of viral escape mutations.

Case report

A previously healthy 26-year-old man presented with signs of an acute HIV-1 infection syndrome [HIV enzyme-linked immunosorbent assay (ELISA) weakly positive; Western blot gp100+, p55+]. His initial plasma viral load was 314 000 HIV-1 RNA copies/ml and CD4 cell count was 726 cells/µl. His last negative HIV-1 ELISA test was in March 2002. A less-sensitive enzyme immunoassay (‘detuned ELISA’) was non-reactive, supporting a recent infection in this individual [5,6].

The study subject did not initiate antiretroviral therapy and was followed closely (Fig. 1a). Fourteen months after the initial presentation the CD4 cell count had dropped to 43 cells/µl and the viral load increased to 2.5 x 10⁶ copies/ml. These results were confirmed 10 days later (Fig. 1a). At this time, the study subject initiated HAART (abacavir plus lamivudine and lopinavir/ritonavir).

A superinfection with a second viral strain as a cause for the increase in viral load [7,8] was excluded by sequencing and clonal sequence analysis of gag and env genes amplified from plasma at baseline and months 9, 12, 13 and 14 post-infection. Comparison of the gag genes over the course of the study revealed that only minor sequence changes occurred, reflecting a predictable degree of natural HIV-1 evolution. Co-receptor tropism and neutralizing antibody activity of the viral envelope was investigated at baseline and at 9 months post-infection using a cell-based infectivity assay [9,10]. All clones of the env gene from the first timepoint displayed R5 tropism, but had changed by 9 months to a dual-mixed phenotype with low X4 usage (Fig. 1d).

No HIV neutralizing antibody activity was detected in the patient’s plasma against autologous or heterologous viruses (APV1–20) at baseline, but some activity was detected against autologous virus and reference clones (NSC/NL4–3/SF162) at 9 months post-infection (Fig. 1c). The neutralization sensitivity of the rapid progressor’s virus was evaluated using plasma from a long-term non-progressor with known broadly neutralizing activity [11]. We found that the baseline virus of the rapid progressor was resistant to neutralization by plasma from the long-term non-progressor, whereas most other heterologous acute viruses tested were sensitive to neutralization by this plasma. These data suggest that the rapid progressor studied was infected with a relatively neutralization-resistant viral envelope, and may partly explain his inability to control viral replication.

Virus-specific CD8 T-cell responses were assessed using flow cytometric quantification of IFN-γ production following stimulation with peptide pools of HIV-1 Gag, Pol, Nef, Env, and Acc (Ypr, Vpr, Vif, Tat, Rev) [7]. HIV-1-specific CD8 T-cell responses were highest at 4 months post-presentation, before the increase in HIV-1 replication, and the dominant HIV-specific CD8 T-cell responses were geared towards Pol followed by Gag (Fig. 1b). The dominant epitope-specific CD8 T-cell
Fig. 1. Full details of study subject. (a) Longitudinal assessment of plasma viral load and CD4 cell count of the study subject, who reached a CD4 cell count of less than 50 cells/μl within 14 months, whereas the plasma viral load increased to $2.5 \times 10^6$ copies/ml. After treatment initiation with HAART the viral load went under the limit of detection and the CD4 cell count increased to 200 cells/μl. (b) IFN-γ response of CD8 T cells was assessed using intracellular cytokine staining in response to (gag, pol, nef, env, acc) at baseline, 4, 6 and 12 months after the first presentation with acute HIV-1 infection syndrome (wide bars), as well as using IFN-γ enzyme-linked immunospot and human leukocyte antigen (HLA)-matched [HLA-A2, -A68, -B14, -B44, -CW5, -CW8] optimal epitopes (narrow bars). The total responses were low and never exceeded 0.5% of CD8 T cells. The highest responses were seen against pol and the HLA-A2-restricted reverse transcriptase epitope YL9. Subdominant responses against the HLA-A68 restricted epitope QV9 in p17 or HLA-Cw8 restricted epitope in p24 TL8 never exceeded 300 spot-forming cells/million. All responses subsequently declined in the context of increasing viremia at the 6 and 12 month timepoints. (c) Neutralizing antibody titers were measured at baseline and 9 months post-infection against autologous virus, a panel of heterologous acute viruses (APV 1–20), neutralization-sensitive reference strains (NL4-3 and SF162), and a neutralization sensitive and resistant clone isolated from the same chronically infected patient (NSC and NSR, respectively). Neutralization titers are expressed as the reciprocal of the plasma dilution that inhibited virus infection by 50% (IC50). Shading indicates a neutralizing antibody titer three times greater than the negative control infection with murine leukaemia virus (aMLV) pseudotyped virus. (d) Co-receptor usage and V3 sequence alignment of selected envelope clones. Recombinant pseudotyped viral particles were produced by co-transfecting HEK293 cells with an envelope expression vector carrying the patient-derived envelope gene and an HIV genomic vector carrying a luciferase reporter gene. The resulting pseudotyped virions were used to infect U87 target cells expressing CD4 and CCR5 or CXCR4. Env clones able to mediate infection of CCR5-positive cells, but not CXCR4-positive cells were designated R5 tropic. Env clones able to mediate infection of both CCR5 and CXCR4-positive cells were designated R5/X4 or dual tropic. (e) Longitudinal alignment of gag and pol sequences show that no escape mutations occur at any timepoint within the targeted epitopes or the flanking regions. ABC, Abacavir; LPV/r, lopinavir/ritonavir; LTNP, long-term non-progressor; PBMC, peripheral blood mononuclear cell; SFC, spot-forming cell; 3TC, lamivudine.
response assessed by IFN-γ enzyme-linked immunoprecipitation [7] was directed against an HLA-A2-restricted epitope in HIV-1 reverse transcriptase (YTAFTIPSI; A2−Y19) and reached 1230 spot-forming cells/10⁶ peripheral blood mononuclear cells after 4 months of infection. Subdominant responses (<300 spot-forming cells/10⁶ peripheral blood mononuclear cells at 4 months) were directed against the human leukocyte antigen Cw8-restricted epitope TPQDNLMTL in p24 (Cw8−TL9) and the human leukocyte antigen A68-restricted epitope QVSNQNPIV in p17 (A68−QV9). These HIV-1-specific CD8 T-cell responses subsequently declined in the context of an increasing viral load to less than 0.15% of CD8 T cells at 6 and 12 months, and responses to individual epitopes became undetectable. Interestingly, sequence analysis of the autologous virus within the targeted epitopes demonstrated that the decline in epitope-specific CD8 T-cell responses was not a consequence of viral sequence evolution, as the autologous viral sequence within the targeted epitope and flanking regions remained conserved (Fig. 1e).

These data demonstrate that rapid progression in HIV-1 can be associated with both weak initial neutralizing antibody responses and the subsequent impairment of CD8-mediated antiviral activity in the absence of viral escape mutations. This report indicates that HIV-1 itself can have a strong direct impact on the dysfunction of adaptive immune responses, emphasizing the need for further research to understand mechanisms of viral immune evasion in the absence of viral sequence variation.

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References


Correspondence

Polymorphisms in the 3′ untranslated region of the fractalkine (CX3CL1) gene and the risk of HIV-1 infection and disease progression

Fractalkine (CX3CL1) is the only natural ligand of the HIV-1 co-receptor CX3CR1 [1,2]. In vitro data support a potent and specific role for CX3CL1 as a blocking factor for HIV-1 infection [3]. No reports so far have assessed whether the CX3CL1 gene is polymorphic. A search of the single nucleotide polymorphisms database from the National Center for Biotechnology Information (www.ncbi.nlm.nih.gov) identified two polymorphic sites located at the 3′ untranslated region (UTR) of the gene, a T to C transition at position 2999 and a T to C transition at position 3042. The 3′ UTR of the gene is a critical region for messenger RNA stability and protein translation [4,5]. In this study we assessed whether these two CX3CL1 genetic variants influence the risk of HIV-1 infection or disease progression among infected individuals in a cohort of adult white Spaniards with CCR5Δ32 wild type.

Our study cohort consisted of 263 white Spaniards; 86 healthy controls, 31 repeatedly exposed but uninfected...
Table 1. CX3CL1 genotype and allele frequencies in the groups analysed.

<table>
<thead>
<tr>
<th>Genotype and allele frequencies</th>
<th>HC (n=86)</th>
<th>EU (n=31)</th>
<th>HIV-1-infected (n=146)</th>
<th>P</th>
<th>HIV-1 UP (n=88)</th>
<th>HIV-1 LTNP (n=58)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>CX3CL1 2999 (T&gt;C)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TT</td>
<td>55 (64%)</td>
<td>21 (70%)</td>
<td>72 (52%)</td>
<td>0.15</td>
<td>2.8 (34%)</td>
<td>22 (40%)</td>
<td>0.81</td>
</tr>
<tr>
<td>TC</td>
<td>27 (31%)</td>
<td>7 (23%)</td>
<td>50 (36%)</td>
<td></td>
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<td>CC</td>
<td>5 (6%)</td>
<td>2 (7%)</td>
<td>16 (12%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Variant allele C</td>
<td>35 (40%)</td>
<td>11 (18%)</td>
<td>82 (58%)</td>
<td>0.03</td>
<td>48 (29%)</td>
<td>34 (31%)</td>
<td>0.70</td>
</tr>
<tr>
<td><strong>CX3CL1 3042 (T&gt;C)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td>TT</td>
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</tr>
<tr>
<td>TC</td>
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<td>8 (27%)</td>
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<td>30 (36%)</td>
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<td>Variant allele C</td>
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<td>12 (20%)</td>
<td>85 (51%)</td>
<td>0.003</td>
<td>48 (29%)</td>
<td>37 (34%)</td>
<td>0.40</td>
</tr>
</tbody>
</table>

EU, Repeatedly exposed to HIV-1 but uninfected individuals; HC, healthy controls; LTNP, long-term non-progressors; UP, usual progressors. The CX3CL1 genotype distribution at positions 2999 and 3042 in control subjects fit the expected Hardy–Weinberg equilibrium. Genotype and allele numbers do not match with patients studied because DNA for CX3CL1 genotyping could not be amplified in some individuals.

individuals, and 146 HIV-1-infected patients. Of these patients, 88 were usual progressors and 58 were long-term non-progressors (LTNP) of over 16 years’ duration. The details and characteristics of this cohort have been extensively described elsewhere [6,7]. The project was approved by the local ethical research committees. Five hundred microlitres of whole blood was used for genomic DNA isolation. The 3’UTR CX3CL1 single nucleotide polymorphisms mentioned above were studied by amplifying a fragment of 242 basepairs and then automatic sequencing of the polymerase chain reaction (PCR) products. Primers from the GenBank sequence BC001163.1 were as follows: 5’-GGTGCGGCAGCTATT-3’ and 5’-ATCCCTGTCATGTCCTGC-3’. PCR was carried out in a final volume of 50 μl, containing 100 ng DNA, 0.2 mM deoxyribonucleotide triphosphate (Roche Diagnostics GmbH, Mannheim, Germany), 0.2 μM of each primer (Sigma Chemical Co., St Louis, Missouri, USA), 3 mM magnesium chloride, 1 U Taq DNA polymerase (GeneCraft GmbH, Lüdinghausen, Germany). The PCR conditions were as follows: 96°C for 7 min, followed by 35 cycles each of 96°C for 30 s, 58.5°C for 30 s and 72°C for 30 s. A final extension step was carried out at 72°C for 7 min. PCR products were visualized on 2% gel stained with ethidium bromide. Amplicons were purified using the QIAquick PCR purification kit. Purified products were automatically sequenced in both directions on an ABI PRISM 310 DNA sequence analyser and following the protocol of the Big Dye Terminator cycle sequencing kit version 3.1 (Applied Biosystems, Foster City, California, USA). Genotype distribution and allele frequencies in the different groups were compared using the χ² test or Fisher’s exact test when necessary. A P value of less than 0.05 was considered significant. The Hardy–Weinberg equilibrium was assessed by the χ² goodness-of-fit test.

Table 1 shows the CX3CL1 genotype and allele distributions in the different groups. Carriage of the CX3CL1 2999C and 3042C variant alleles was more frequent in HIV-1-infected patients than in uninfected subjects: P = 0.03 for 2999C, and P = 0.003 for 3042C. None of these allelic variants influenced disease progression because they were equally represented in both usual progressors and LTNP. These new data merit consideration, however, because the associations that we found are based only on allelewise comparisons and not on genotype-wise comparisons. As pointed out elsewhere, associations based on a single contrast are less likely to be replicated in further studies than those based on more contrasts [8], therefore the data must be treated with caution. The rationale for studying CX3CL1 is that the complex chemokine–chemokine receptor is currently considered to be a functional unit, thus the ability and availability of a particular co-receptor to bind HIV-1 may be mediated by chemokine binding. In this regard our results suggest that the CX3CL1 2999C and 3042C allele may have a modulating effect on the disease, and the higher prevalence of this mutant allele in HIV-1-infected patients may be a predisposing factor to infection. These findings emphasize that the CX3CR1/CX3CL1 axis may influence HIV-1 disease transmission, as has been suggested by the finding of an increased CX3CL1 expression in several cell types in T-cell zones of lymph nodes from HIV-1-infected patients [9]. Moreover, CX3CL1 is considered to be a T helper type 1 chemokine [10,11] as well as IFN-γ, IL-1 and TNF-α. CX3CL1 therefore plays an important role in a T helper 1-type immune response as the amplification circuit of polarized T helper type 1 cells [12]. Given the minor in-vivo role of CX3CR1 in the early phases of HIV-1 infection, it seems plausible that the effects of CX3CL1 on HIV-1 transmission are a result of its immune properties rather than CX3CR1 blockade.

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Mycobacterium sherrisii: a new opportunistic agent in HIV infection?

*Mycobacterium sherrisii*, a recently described mycobacterial species closely related to *Mycobacterium simiae*, has previously been implicated in a pulmonary infection [1]. We report the first case of disseminated *M. sherrisii* infection in a severely immunocompromised patient.

A 53-year-old man living in Africa for 30 years was transferred to France with a fever and general weakness of several months’ duration. He had tested positive for HIV infection in 2003 while hospitalized for pulmonary tuberculosis. He was then lost to follow-up until 2005, when HAART was started (nevirapine, lamivudine and stavudine).

On admission, he was wasted and had a Karnofski index of 70%. The CD4 cell count was 19 cells/µl (3.2%) and the viral load was 460 copies/ml. A computed tomography scan showed bilateral ground-glass opacities and signs of tubercular sequelae in the upper right lobe. An abdominal computed tomography scan showed...
abdominal adenopathies and mesenteric infiltration. An ascitic fluid sample contained a lymphocytic exudate. Recurrent tuberculosis was initially suspected, and he was treated empirically with isoniazid, ethambutol, rifampicin and pirazinamide. A few weeks later five sputum samples cultured for between 6 and 16 days, a peritoneal effusion sample cultured for 11 days, and two blood cultures cultured for 13 and 19 days yielded atypical mycobacteria, initially identified as *M. simiae*. His antimycobacterial treatment was switched to clarithromycin, rifabutin, moxifloxacin and amikacin, based on sensitivity tests and published data. The patient’s condition improved when this combination was started; his poor immunological status might explain the initial inefficacy. These two cases suggest that antibiotic susceptibility testing correlates poorly with clinical efficacy in *M. sherrisii* infection.

Molecular biology-based methods have identified new emerging pathogens that were incorrectly classified. The identification of *M. sherrisii* broadens the spectrum of atypical mycobacteriosis complicating immunodepression. HAART can restore antimycobacterial immunity, but relatively slowly [4,5] and with the risk of an immune restoration syndrome [6] that further complicates the management.

In conclusion, we report the first documented case of disseminated *M. sherrisii* infection, in an HIV-infected patient. The optimal treatment is unclear, as drug susceptibility in vitro may not always predict clinical efficacy.

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