Research Letters

AIDS 2001, **15**:799–817

Differing reverse transcriptase mutation patterns in individuals experiencing viral rebound on first-line regimens with stavudine/didanosine and stavudine/lamivudine

Recent data have suggested that zidovudine-associated resistance mutations at 215Y and 41L may be observed in zidovudine-naive individuals receiving therapy with stavudine-based regimens [1]. Importantly, these mutations may confer some cross-resistance to other non-thymidine nucleoside analogues such as abacavir [2] and posibly other nucleoside analogues. Data from studies involving zidovudine-based regimens suggest that mutation patterns at viral rebound may differ when lamivudine is the co-therapy compared with when didanosine is the co-therapy [3,4]. The type and frequency of these different nucleoside analogue mutations (NAM) observed when stavudine is combine with didanosine or lamivudine is not established.

Over 700 Vircogen genotypes (Virco, Belgium) have been performed at the Chelsea and Westminster Hospital over 18 months of availability. We extracted data on reverse transcriptase mutations on patients receiving their first ever regimen containing stavudine plus either didanosine or lamivudine.

Of 47 patients identified, 24 were on didanosine and 23 on lamivudine as co-therapy. The CD4 cell counts, viral load and duration of therapy were similar between the groups (Table 1). Most patients were receiving a third agent. In the stavudine/lamivudine group, 18 patients were receiving a protease inhibitor (PI) and one a non-nucleoside reverse transcriptase inhibitor (NNRTI). In the stavudine/didanosine group four patients were receiving a PI and 11 an NNRTI. NAM (at codons 41, 67, 70, 210, 215 and 219) were observed in 26 (55%) samples. The mean number of nucleoside type reverse transcriptase mutations (see Table 1) was didanosine 1.67 and lamivudine 2.04, with no nucleoside reverse transcriptase inhibitor mutations present in four (17%) didanosine and one (4%) lamivudine recipient. Only nine (19%) patients (six on didanosine, three on lamivudine) had three zidovudine-type mutations or more. The 184V mutation was observed in one (4%) didanosine and 22 (96%) lamivudine recipients ($P < 0.001$ by chi-squared test) and was the sole mutation in nine (39%) lamivudine patients. The 151M multi-nucleoside resistance mutation was observed in only one individual in the didanosine group. Mutations 65R (two patients), 74V (two) and 75T (one) were only observed with didanosine. Although 41L occurred at a similar frequency (seven didanosine and five lamivudine recipients), 215Y/F was more common with didanosine [11 (46%) versus five (22%) patients], whereas 70R was more common with lamivudine (one didanosine versus six lamivudine). Among the 11 patients who received an NNRTI with stavudine/didanosine, the seven nevirapine recipients all had NNRTI mutations, including four with 181C, two with 103N and one with 190A, implying that despite the absence of zidovudine in a regimen the K103N mutation may be observed.The four efavirenz recipients all had 103N, one sample also having 181C. The one nevirapine plus stavudine/lamivudine recipient had a 188L mutation only.

In conclusion, NAM were commonly observed in this cohort of zidovudine-naive, stavudine-treated patients who were receiving their first ever antiretroviral regimen. As no pretherapy samples were available, it is possible that some of these mutations may have derived from transmitted zidovudine resistance. However, other groups have also observed the emergence of NAM during stavudine-based therapy [1,5,6], suggesting that these mutations may provide replicative advantage for HIV in the presence of stavudine. As with zidovudine-based therapy, in which the 70R mutation is not commonly observed during combination with didanosine, but is observed during lamivudine co-therapy [3], the choice of co-nucleoside may influence the mutational pattern observed with stavudine. Patterns observed in this cohort are consistent with zidovudine patterns. Importantly, no nucleoside analogue mutations at rebound are observed in 17% during didanosine co-therapy, whereas 184V is almost invariable when lamivudine is used. Despite a considerable duration of exposure to stavudine (mean 18 months), 45% of patients did not have NAM, suggesting that stavudine, zidovudine and abacavir would remain future treatment options for these individuals. Similarly, only 10 patients (nine with three or more zidovudine mutations plus one with 151M) had mutation patterns consistent with a likely diminished response to abacavir [2].

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Received: 6 November 2000; accepted: 30 January 2001.

Table 1. Demographics, treatment markers and reverse transcriptase mutations observed during first-line stavudine-based regimens with lamivudine or didanosine.

Regimen	Duration (months)	Mean VL	Mean CD4 cells/mm^3	Mean no. mutations	41L	62L	65R	67N	70R	74V	75T	184V	210W	215Y	219E	333E	151M
Stavudine/ lamivudine	20.217	9275.8	350.22	2.0435	5	1	0	3	6	0	0	22	1	5	2	2	0
	Range 3–36	Range 80–43, 340	Range 64–1101	Range 0–5													
Stavudine/ didanosine	15.167	22 379	432.92	1.6667	7	0	2	4	1	2	1	1	5	11	3	1	1
	Range 1–37	Range 234–159, 117	Range 61–1039	Range 0–4													

VL, Viral load in copies/ml.

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Polymerase chain reaction for Y chromosome to detect semen in cervicovaginal fluid: a prerequisite to assess HIV-specific vaginal immunity and HIV genital shedding

Ensuring that cervicovaginal secretions obtained from sexually active women are free of semen is essential to avoid misinterpretation of the data and accurately assess the immune response in the female genital tract. Similar precautionary measures should be undertaken when analysing genital shedding of HIV in infected women. The presence of semen in cervicovaginal secretions (CVS) may be assessed by the immunochemical detection of semen-derived components by immunocapture assays, including prostatic acid phosphatase, prostatic-specific antigen (PSA) [1] and seminal vesicle-specific antigen [2]. However, the latter methods may lack specificity and sensitivity. The present study was carried out to validate a highly sensitive polymerase chain reaction (PCR) for the Y chromosome in the cellular fraction of CVS for detecting contaminating semen in female genital fluids.

Two hundred and thirteen unselected women attending the National Réference Center for Sexually Transmissible Diseases and AIDS in Bangui, Central African Republic participated in the study. We followed the ethical recommendations of the Ministry of Health of the Central African Republic, and verbal informed consent was obtained from all participants. Women entering the study underwent general, genital and pelvic examination, during which CVS were collected as described below. A 7 day follow-up appointment was arranged for all women, and appropriate treatment was provided free of charge for any treatable sexually transmitted infection syndrome or genital pathogen diagnosed. CVS were collected by a standardized non-traumatic 60 s vaginal washing with 3.0 ml of phosphate-buffered saline (PBS), as previously described [3]. The cellular fraction and the cell-free fraction of CVS were separated by centrifugation at 1000 ***g*** for 10 min and were kept frozen at −80°C until processing.

The detection of PSA and PCR amplification of DNA of the Y chromosome were performed in parallel in all CVS samples collected. The detection and quantitation of PSA were performed in 150 µl of the acellular fraction of CVS using an immunoenzymatic assay with a threshold of positivity of 0.1 ng/ml (PSA IMX System, Abbott Laboratories, Abbott Park, Chicago, IL, USA). The cutoff for the presence of PSA antigen in cervicovaginal fluid was 0.4 ng/ml, determined as the mean + 2 standard deviations (SD) of the values obtained with this assay in 150 µl of CVS obtained from 30 healthy childbearing-aged HIV-seronegative Caucasian women claiming to be not sexually active at the time of sampling and recruited as controls. For the PCR of Y chromosome, DNA was extracted from the cellular pellet of CVS using the QIAamp DNA kit, according to the manufacturer's recommendations (Qiagen AG, Basel, Switzerland). One microgram of extracted DNA was processed for Y chromosome DNA amplification, by means of a single PCR using as primer set, SRY3F and SRY3R, specific for a 229 bp region in the sex-determining region (SRY), a gene located on the short arm of the Y chromosome, as described [4]. In order to control the quality of extracted DNA and the lack of PCR inhibitors, the ubiquitous β-globin gene was amplified by PCR.

A total of 213 women (mean age 27 years; range 15–48) were eligible for enrollment. None refused to participate in the study. The median age of first sexual intercourse was 16 years, with a median of two (range 1–8) reported lifetime partners. Forty-four women were found to be seropositive for HIV-1 (20.6%). DNA extracted from the cellular pellet of CVS was tested positive by PCR for the β-globin gene in 204 samples (96%). The nine cervicovaginal samples tested negative for the β-globin gene, suggesting poor conservation or a low amount of DNA in these samples, were excluded from the analysis. When tested for the presence of the PSA antigen, 41 of the 204 β-globin-positive CVS samples (20%) showed an optical density above the cutoff of positivity. The mean concentration of PSA antigen ± SD was 19.9 ± 20.0 ng/ml, with important differences among CVS samples. The concentrations of PSA thus ranged from 0.4 to 2 ng/ml in nine samples; from 2.1 to 10 ng/ml in 11 samples; from 10.1 to 50 ng/ml in 11 samples; and were above 50.1 ng/ml in 10 samples (interquartile range 2.9–39.9). The cellular fractions of the 204 β-globin-positive CVS were further tested for the SRY gene. Seventy-three (36%) samples gave an amplicon as a unique and clearly distinguishable band of 129 base pairs, and were considered to be positive for the Y chromosome. All PSA-containing CVS (n = 41, 20% of CVS) were also positive for SRY DNA. Thirty-two CVS samples (16%) were only positive for the presence of the Y chromosome, with no detectable PSA. The number of semen-containing CVS detected by the Y PCR was significantly higher than the number of semen-containing CVS detected by PSA detection ($P < 0.001$). The remaining 131 (64%) cervicovaginal samples were both PSA and Y chromosome negative.

In the present study, we demonstrate that the PSA immunocapture assay, one of the most sensitive, specific and commonly used immunoenzymatic assays available to detect semen in the CVS collected from women practising unprotected sexual intercourse [5], did not identify 32 out of 73 (44%) Y PCR-positive CVS. Our findings show that cervicovaginal secretions from sexually active women may contain semen unrecognized by conventional immunoenzymatic assay

used to detect semen components. The detection of semen components in female genital secretions after peniovaginal intercourse depends on the clearance of the semen components and on the sensitivity of the methods used. Although the clearance of semen-associated DNA deposited in the vagina is unknown, it is likely that the DNA protected in the nucleus of spermatozoids or male gamete precursors is relatively stable in the lower female genital tract. One may hypothesize that the clearance of semen-associated DNA in the female genital tract is lower than that of soluble molecules such as PSA, in agreement with our observation that the Y chromosome was always amplified when PSA was detected in the CVS of sexually active women. The data suggest that the detection of male DNA with a higly sensitive and specific procedure such as Y PCR constitutes a method of choice to detect semen traces in female genital secretions.

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Sponsorship: This work was supported by the Institut National de la Santé et de la Recherche Médicale, the Université Pierre et Marie-Curie (Paris VI), and the Agence Nationale de Recherches sur le SIDA. N.C. is recipient of a scholarship of the Ministère de l'Education Nationale, de la Recherche et de la Technologie, Paris, France.

Received: 1 December 2000; revised: 26 January 2001; accepted: 30 January 2001.

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Jejunal cytokine response in AIDS patients with chronic cryptosporidiosis and during immune reconstitution

Chronic cryptosporidiosis in AIDS patients was formerly a debilitating illness that frequently resulted in death [1]. With effective antiretroviral therapy, many patients experience complete a resolution of symptoms and suppression [2], or elimination of the parasite [3,4]. Although a previous study demonstrated an increase in CD4 T cell numbers in the colonic mucosa of AIDS patients after short-term combination therapy [5], little information exists on the effects of antiretroviral combination therapy on local cytokine production by the gastrointestinal tract in AIDS after highly active antiretroviral therapy (HAART). To our knowledge no data exist regarding the local intestinal cytokines associated with the control of opportunistic infection or after immuno-reconstitution in AIDS.

We previously demonstrated that the resolution of *Cryptosporidium parvum* infection in immunocompetent adults is associated with the production of IL-15. In contrast, infection in sensitized individuals is controlled by the production of γ-IFN [6]. The objective of this study is to describe the local cytokine response in the intestines in AIDS patients with chronic cryptosporidiosis, and to document the changes in cytokine profiles noted when cryptosporidiosis resolves in response to HAART.

Seven AIDS patients with AIDS, chronic diarrhea, cryptosporidiosis, and *C. parvum* oocysts in their stool participated in the study. A baseline evaluation included a medical examination, the determination of circulating CD4 T cell numbers and HIV viral load. The baseline mean CD4 T cell count was 25 ± 28 cells (range 5–75) and the mean viral load was 5.82 (log). All patients were antiretroviral experienced. At baseline, two patients were receiving monotherapy with stavudine (biopsied before the availability of protease inhibitors), two patient were receiving combination regimens but with antiviral failure, and two had been heavily pretreated and were off antiviral therapy at the time of enrolment.

Subjects were asked to provide 24 h stool collections for oocyst quantitation as well as to undergo endoscopy with jejunal biopsy. On the basis of previous antiviral exposure, the antiretroviral regimens were modified. Patients were started on azithromycin 600–1200 mg a day and paromomycin 2 g a day (in two to four doses) [7]. Four to 24 weeks later, patients were reassessed for response. In those who demonstrated clinical improvement, the endoscopy was repeated and stools examined for oocysts. Intestinal biopsy specimens were immediately fixed in diethyl pyrocarbonate-treated paraformal-

Table 1. Cytokine responses as determined by in-situ hybridization in jejunal biopsy specimens observed in seven patients with chronic cryptosporidiosis.

Response	Patient	Timing of biopsy	Diarrhea	AFB	DFA	Antiretroviral regimen	CD4 cell count	HIV viral load	IFNγ	IL-15	IL-4	TGFβ
Non-responders	1	Baseline	Present	+	13 600	Stavudine	7	ND	–	–	–	–
	2	Baseline	Present	++	2900	Stavudine	75	ND	–	–	–	+
	3	Baseline	Present	++	27 000	Stavudine, indinavir, lamivudine	18	128 633	–	–	–	+
Partial responder	4	Baseline	Present	+	N/A	Didanosine, nelfinavir, hydroxyurea	9	ND	ND	ND	ND	ND
		Week 4	Improved	+	14	Didanosine, nelfinavir, hydroxyurea, stavudine	22	192 470	+	+	+	+
Responders	5	Baseline	Present	+	4	None	9	275 204	–	–	–	–
		Week 4	Resolved			Nevirapine, saquinavir, ritonavir	N/A	N/A	–	+	–	–
		Week 12	Resolved	–	–	Nevirapine, saquinavir, ritonavir	66	157 280	+	–	++	–
	6	Baseline	Present	+	8800	Ritonavir, saquinavir, nevirapine	55	1 825 790	–	–	–	–
		Week 24	Resolved	–	ND	Amprenavir, abacavir, didanosine	112	49 990	–	++	ND	–
	7	Baseline	Present	+	2500	None	5	445 950	–	–	–	–
		Week 16	Resolved	–	ND	Lamivudine, efavirenz, stavudine	145	< 400	++	–	++	–

AFB, Acid-fast bacillus stain; DFA, direct fluorescent antibody (oocysts per ml $\times 10^3$); TGFβ, transforming growth factor beta.
Three patients resolved infection and were re-studied at 4–24 weeks after the modification of antiviral therapy.

dehyde (60 min, room temperature), washed in diethyl pyrocarbonate-treated phosphate-buffered saline, and stored in 70% ethanol until sectioning. Cytokine responses were assessed by in-situ hybridization with [35S]-labelled riboprobes for IFNγ, transforming growth factor beta (TGFβ), IL-15, and IL-4 using methods previously described [6,8]. TGFβ was the only cytokine identified in the baseline biopsies and was found in two out of six patients studied.

Out of the seven patients, three resolved their diarrhea, and one improved but did not resolve the infection after modifications in antiviral therapy as outlined in Table 1. In the three responders, the CD4 T cell counts increased a mean of 85 cells (range 57–140), and the viral load decreased by a median of 1.41 log (range 0.24–3.04). Patient five demonstrated an increase in the IL-15 signal at week 4, which was followed by increases in IFNγ and IL-4 at week 12. Patient six demonstrated IL-15 weakly at week 24. Finally, patient 7 demonstrated increases in both IFNγ and IL-4 at week 16. Patient 4 demonstrated some clinical improvement at week 4, and was found to have messenger RNA for IFNγ, IL-15, TGFβ and IL-4.

These data suggest that several intestinal cytokines are expressed in response to chronic cryptosporidiosis in patients who are receiving antiviral therapy. TGFβ stimulates B cells to switch to the production of IgA. It also participates in the restoration of damaged epithelium. Its expression in chronic infection is consistent with our previous observation of expression in normal volunteers shedding oocysts, and probably reflects a response to counteract ongoing injury. By contrast, mRNA for either IL-15 or IFNγ and IL-4 could be identified in biopsies from all three patients who had resolved cryptosporidiosis in response to antiretroviral therapy. In previous studies, we noted IFNγ and IL-4 as part of the response of sensitized HIV-negative normal volunteers to *C. parvum*, whereas the expression of IL-15 was associated with the response in naive volunteers. The numbers of patients and biopsies studied here is insufficient to determine the timing. However, the sequential expression in subject 5 suggests that perhaps AIDS patients develop a local naive and then a memory response. For now, we can conclude that in AIDS patients who are undergoing immune reconstitution, intestinal cytokine expression increases the associated with the resolution or improvement of *Cryptosporidium* infection. The patterns of cytokines expressed are similar to those noted in self-limited infection in normal volunteers. There is thus evidence of local as well as systemic immune reconstitution.

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Grant Support: US Environmental Protection Agency (#CR-819814 and #CR-824759), National Institutes of Health R01 AI 41735-01 and RR-02558 and the Food and Drug Administration (FD-U-001621-01).

Received: 18 October 2000; accepted: 30 January 2001.

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Long-term safety and efficacy of nevirapine, stavudine and lamivudine in a real-world setting

Recent studies have demonstrated the efficacy of protease inhibitor-sparing regimens including non-nucleoside reverse transcriptase inhibitors (NNRTI) in treatment-naive patients [1–3]. Long-term side-effects of protease inhibitors, including lipodystrophy and hyperlipidemia, as well as their often complicated dosing regimens, have led to considerable interest in the use of the NNRTI as first-line therapy. To date, however, only limited clinical trial data have been presented that demonstrate long-term virological suppression with this class of agent [4].

The purpose of this community-based study, therefore, was to investigate the long-term safety and efficacy of a regimen of nevirapine, stavudine and lamivudine in a real world setting. Twenty-six treatment-naive patients were prospectively enrolled in a study of a highly active antiretroviral therapy regimen of nevirapine, stavudine and lamivudine at a community-based clinic. The median age of the group was 43 years (range 25–66), 25 of the patients were men. The median baseline viral load was 38 138 copies/ml (range 4846–212 852 copies/ml), and the median baseline CD4 cell count was 360 cells/mm^3 (range 98–920 cells/mm^3). The mecian length of time to follow-up was 31 months (range 8–40 months). Ninety-two per cent (24/26) had viral loads of less than 50 copies/ml at their last visit, with one patient having a viral load of 657 copies/ml, and one being off medication. This group of patients maintained a viral load of less than 50 copies/ml for a mean of 19 months (range 7–38 months). Thirteen of these patients had been virologically suppressed for over 2 years at latest follow-up. The median CD4 cell count at the last follow-up was 575 cells/mm^3. The regimen of nevirapine, stavudine and lamivudine, therefore, provided a potent and durable response in this population.

This regimen also proved to be remarkably well tolerated, with reports of two cases of peripheral neuropathy secondary to stavudine, but no nevirapine-associated clinical side-effects. Some slight elevations in transaminase levels were observed after initiating therapy; serum glutamic-oxaloacetic transaminase increased from 26.6 to 28.7 U/l, and serum glutamate-pyruvate transaminase increased from 25.8 to 38.6 U/l. However, transaminase levels did not increase in any individual to greater than two times the upper limit of normal at any point, and were not clinically relevant.

This simple nevirapine-based combination regimen of three tablets twice a day, with no food restrictions, provided durable virological suppression for up to 38 months in a community-based cohort of treatment-naive patients. Highly active antiretroviral therapy containing nevirapine can, therefore, be effectively used as a long-term protease inhibitor-sparing regimen. No loss of virological control was seen in this cohort, reducing concerns for the emergence of NNRTI-associated resistance over time. This study was not designed to look at lipodystrophy. However, lipid abnormalities and fat redistribution have not been associated with nevirapine; indeed, nevirapine has been associated with improvements in hyperlipidemia and lipodystrophy after a switch from protease inhibitors [5], adding additional appeal for regimens such as the one described here. The lack of both short- and long-term side-effects with this potent regimen also makes it a very attractive alternative for treatment-naive subjects. The improved quality of life associated with a simple regimen and absence of side-effects is likely to impact positively on drug adherence, and could explain the durability of this nevirapine-based antiretroviral regimen in such a high proportion of patients.

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Received: 19 September 2000; revised: 5 January 2001; accepted: 10 January 2001.

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Indinavir and systemic hypertension

The introduction of antiretroviral therapy that includes a protease inhibitor (PI) has changed dramatically the clinical perspectives for HIV-infected individuals [1]. Because the risk of HIV mortality is reduced, the importance of long-term side-effects associated with the use of PI has become a relevant issue [2,3]. Indinavir, one of the most widely used PI, has been associated with renal calculi and nephropathy [4,5]. Blood hypertension, as a consequence of indinavir treatment, has not been reported. The aim of this retrospective study was to evaluate the frequency of this side-effect in a cohort of HIV-infected patients receiving indinavir; individuals treated with other PI were used as a reference group. The study population was based on a cohort of HIV-infected patients receiving longitudinal care through the outpatient unit of the Division of Infectious Diseases of Padua, Italy, between January 1997 and June 2000. Inclusion criteria consisted of a duration of PI treatment of at least 6 months and no previous therapy with PI. Individuals with previous hypertension, and confirmed non-compliance (self-reported or drug-monitored) were excluded. According to an internal protocol, patients were followed at monthly clinical visits, at which time blood samples were drawn and blood pressure was taken. Biochemical parameters and urine analysis were monitored every 4 weeks, CD4 cell counts and HIV-RNA levels were monitored every 12 weeks. In the subset of patients in which hypertension was recorded, at least three repeated measurements of blood pressure were taken during a one month period. In addition, 24 h urine collection in order to check for creatinine clearance, protein and glucose excretion, the renin–angiotensin system, together with renal ultrasonography and a Doppler flow study were performed. Hypertension was defined as systolic blood pressure of 140 mmHg or higher, diastolic blood pressure of 90 mmHg or higher, or both [6].

A total of 198 patients were evaluated. Of these, five patients were excluded for the presence of hypertension at baseline, nine for non-compliance and three for lack of follow-up. A study population including 181 patients was thus observed; during a median follow-up period of 34 months (range 6–56), sixty-seven patients (37%) maintained the initial highly active antiretroviral therapy regimen for a median time of 26 months (7–45), whereas 114 patients (63%) changed their highly active antiretroviral therapy, with a median number of regimens of two (2–6). Indinavir was used in 104 patients (group 1) and other PI (nelfinavir, saquinavir, and ritonavir) were used in 77 patients (group 2), which was considered the control group. The baseline characteristics of the two patient groups were well matched for all the considered parameters (Table 1). At study entry both the mean systolic pressure and the mean diastolic pressure were similar in the two groups: 125 mmHg (110–130, SD $\pm$ 10.7) and 81 mmHg (60–85, SD $\pm$ 6.1) in group 1 versus 126 mmHg (105–135, SD $\pm$ 12.0) and 82 mmHg (70–85, SD $\pm$ 6.6) in group 2 (no significant differences were found using the Mann–Whitney test).

During the study period, 31 patients experienced stage 1 or greater blood hypertension: all patients belonged to group 1. The mean systolic pressure was 136 mmHg (105–180, SD $\pm$ 17.8) and the mean diastolic pressure was 91 mmHg (60–120, SD $\pm$ 12.3) in group 1 compared, respectively, with 125 mmHg (105–138, SD $\pm$ 13.0; $P < 0.0001$ Mann–Whitney test) and 80 mmHg (70–88, SD $\pm$ 7.5; $P < 0.0001$) in group 2. In the 31 patients with hypertension, the mean systolic pressure was 153 mmHg (120–180, SD $\pm$ 15.8) and mean diastolic pressure was 100 mmHg (95–120, SD $\pm$ 6.2) compared with baseline values of 120 and 80 mmHg, respectively, ($P < 0.0001$ Wilcoxon rank signed test) (Fig. 1). In six patients a stage 3 hypertension was recorded, in five a stage 2, and in 20 patients a stage 1. The proportion of cases with hypertension was higher in men than in women (M : F: 9 : 1), whereas no

Table 1. Baseline characteristics of the study population.

	All subjects	Group 1	Group 2
Number	181	104	77
Median age in years (range)	39 (27–74)	40 (29–74)	38 (27–68)
Centers for Diseases Control and Prevention stage			
A (%)	4 (2)	2 (2)	2 (3)
B (%)	79 (44)	45 (43)	34 (44)
C (%)	98 (54)	57 (55)	41 (53)
Modality of exposure to HIV			
Intravenous drug users (%)	77 (42.5)	46 (44)	31 (40)
Homo/bisexual contact (%)	66 (36.5)	37 (36)	29 (38)
Heterosexual contact (%)	38 (21)	21 (20)	17 (22)
Mean CD4 cell (± SD) count/μl	195 (± 193)	173 (± 208.4)	225 (± 166.5)
Mean HIV-RNA (± SD) $\log_{10}$/ml	5.4 (± 5.5)	5.4 (± 5.5)	5.3 (± 5.5)
Treatment naive patients (%)	75 (41)	41 (39)	34 (44)
Current reverse trascriptase inhibitor			
Zidovudine/lamivudine (%)	56 (31)	33 (32)	23 (30)
Stavudine/lamivudine (%)	95 (52)	55 (53)	40 (52)
Others (%)	30 (17)	16 (15)	14 (18)
Blood pressure in mmHg[a]			
Mean systolic (± SD)	122 (± 11.1)	125 (± 10.7)	126 (± 12.0)
Mean diastolic (± SD)	78 (± 6.8)	81 (± 6.1)	82 (± 6.6)

Comparisons were made between data on subjects in group 1 and group 2 by unpaired Student's *t*-test (no significant differences were found).
[a]No differences in blood pressure between the two groups were found (Mann–Whitney test).

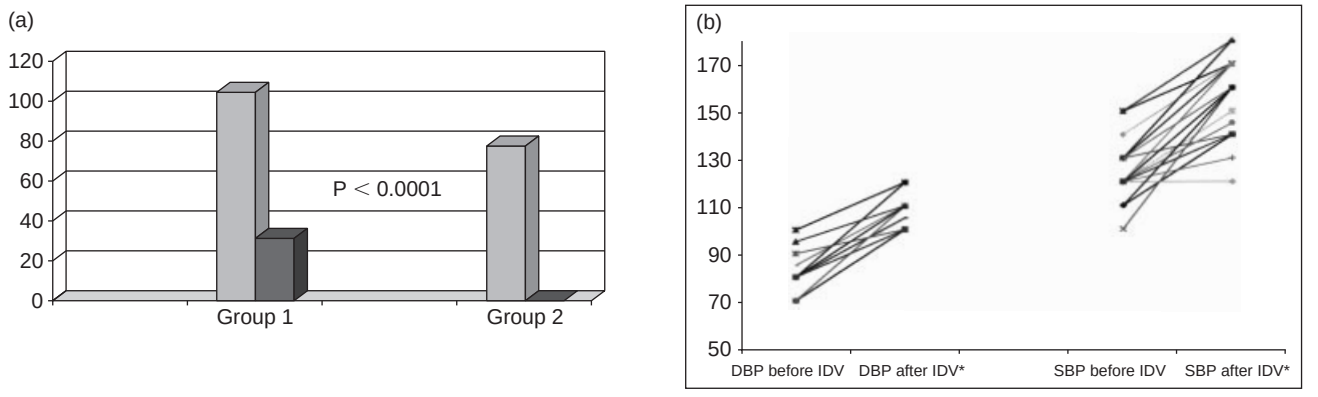

Fig. 1. (a) Cases of hypertension. □ Recruited patients; ■ patients with hypertension. (b) Mean values of blood pressure (diastolic and systolic) in patients with hypertension before and after indinavir therapy. DBP, Diastolic blood pressure; IDV, indinavir; SBP, systolic blood pressure; * $P < 0.0001$, Wilcoxon signed rank test.

significant differences were observed between this subgroup of patients and group 2 in terms of age, CD4 cell count, HIV-RNA levels and type of nucleoside reverse transcriptase inhibitors used in the regimens. No significant changes in renal function were noted, whereas a positive family history for essential hypertension was reported in 18 out of 31 patients (58%).

Blood hypertension was effectively controlled with antihypertensive drugs in 18 patients, in nine indinavir was withdrawn (hypertension recovered in four, whereas it persisted in five cases), and in four patients specific therapy was refused because of mild hypertension.

This retrospective analysis showed that indinavir-containing regimens are significantly associated with blood hypertension. The pathogenesis of this side-effect remains unexplained. Further studies are needed to elucidate both the potential role of the prolonged use of previous antiretroviral therapies and the effects of a re-challenge with indinavir therapy. In addition, epidemiological variables such as obesity, alchohol,

cigarette smoking, and the use of non-steroidal anti-inflammatory agents should be investigated.

Furthermore, it is of interest that more than half of our 31 patients had a positive family history of hypertension. We could speculate that indinavir, at least in some cases, may trigger latent hypertension, rather than directly cause this effect. These data are in agreement with the absence of renal abnormalities and the normal renin–angiotensin system found in our patients. Although the retrospective nature of the study did not allow us to confirm definitively the relationship between indinavir and hypertension, an important point emerges from this analysis: blood pressure needs to be carefully monitored in regimens that include indinavir. This is particularly important in patients with hypertension or a family history of hypertension, in whom indinavir should be considered as a second line PI antiretroviral therapy.

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Received: 4 January 2001; accepted: 30 January 2001.

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Osteopenia in HIV-infected patients: is it the disease or is it the treatment?

Bone loss is a normal feature of aging. Accelerated bone demineralization is a multifactorial process; recent reports have suggested that HIV infection, perhaps in association with protease inhibitor (PI) therapy, is an additional risk factor for osteopenia [1,2]. However, changes in bone mineral metabolism, bone histomorphometry or bone mineral density had been described in HIV-infected patients before the era of highly active antiretroviral therapy (HAART) [3–5].

The aim of this study is to define the role of HIV infection and antiretroviral therapy in the development of bone mineral loss. A cross-sectional study was performed with the following patients enrolled: 80 HIV-infected patients [58 men, 22 women, mean age (SD) 41 years ($\pm$ 8)]; 26 did not receive treatment; 37 were on HAART that included a PI; 17 were on HAART without a PI (two nucleoside reverse transcriptase inhibitors plus one non-nucleoside reverse transcriptase inhibitor). One hundred healthy seronegative adults matched by age and sex served as controls. Patients with known factors of osteopenia were excluded (recent history of extended bed rest, previous diagnosis of metabolic bone disease, renal insufficiency, hepatic failure, diabetes mellitus or previous diagnosis of other endocrine disease, moderate or severe nutritional alteration, and severe alcohol consumption).

Hologic QDR-4500 SL dual energy X-ray absorptiometry was used to determine the bone mineral density (BMD) of the lumbar spine (L1–L4) and proximal femur, with T and Z scores (T score represents a SD in BMD within the mean of the population at 30; Z score represents a SD in BMD within the mean of the same age and sex group). Using World Health Organization definitions of osteopenia and osteoporosis, we classified the patients into the following categories: normal: T score greater than -1; osteopenic: T score from -1 to -2.5; and osteoporotic: T score less than -2.5.

Non-parametric statistical tests (Kruskal–Wallis) were used to compare Z scores for spinal and hip BMD between groups. Normally distributed variables were compared using an analysis of variance. Chi-square and Fisher's exact test were used for categorical variables. Pearson's correlation coefficient was used to evaluate weight, body mass index, duration of therapy, CD4 cell count, and viral load in relation to BMD.

The characteristics of the 180 subjects included in the study and its most relevant results are summarized in Table 1.

No differences were found in HIV-infected patients, irrespective of the treatment or type of treatment; however, the HIV-infected patients have lower BMD compared with healthy adults; the difference in BMD Z score between healthy adults and HIV patients was: in the lumbar spine: -0.43 [$P = 0.001$; 95% confidence interval (CI) -0.7 to -0.2]; in the femoral neck: -0.68 ($P = 0.0001$; 95% CI -0.91 to -0.44).

BMD was not correlated with HIV categories, viral load, CD4 cell count, duration of therapy, or the

Table 1. Characteristics of the 180 patients included in the study.

	HIV-negative (N = 100)	HIV-positive naive (N = 26)	HIV + PI (N = 37)	HIV + NNRTI (N = 17)
Age (years)[a]	40 ± 6	36 ± 10	44 ± 10	42 ± 12
Body mass index (kg/m^2)[a]	23 ± 2	22 ± 4	23 ± 3	23 ± 2
% of patients CDC group A	–	42	27	64
% of patients with lipodystrophy	–	4	54	23
Median lumbar spine BMD	1.064	1.044	0.956	0.968
Median *Z* score				
Lumbar spine (L1–L4)	−0.15	−0.40	−0.68	−0.80
Femoral neck	−0.19	−0.97	−1.27	−0.86
Trochanter	−0.16	−0.62	−0.79	−0.70
% of subjects with osteopenia	25	58	70	76
% of subjects with osteoporosis	5	11	30	18

[a]Values are mean ± SD.
BMD, bone mineral density; NNRTI, non-nucleoside reverse transcriptase inhibitors; PI, protease inhibitors.

presence of lipodystrophy, and was correlated with weight and body mass index ($r = 0.3$; $P = 0.02$).

In relation to the potential role of individual antiretroviral agents on decreased BMD, we found that patients exposed to indinavir (17 patients, as the only PI used) have lower *Z* scores in the femoral neck compared with treated patients who have not been exposed to indinavir (−1.4 versus −0.7; $P = 0.02$).

Osteopenia was present in 25% of healthy adults and in 67.5% of HIV-infected patients [$P = 0.00001$; odds ratio (OR) 6.2; 95% CI 3.1–12.6]. Osteoporosis was present in 5% of healthy adults and in 21.2% of HIV-infected patients ($P = 0.00009$; OR 5.1; 95% CI 1.7–18.5). Differences in osteopenia or osteoporosis did not reach statistical significance among the HIV patient groups.

In our study, a decrease in BMD and a higher rate of osteopenia and osteoporosis was found in HIV-infected patients compared with non-HIV patients. No clear association was found in relation to the use of HAART or the type of HAART used.

Studies made before the HAART era and a more recent study obtained similar conclusions [3–6]. The hypothesis that the systemic activation of T cells *in vivo* leads to an osteoprotegerin ligand-mediated increase in osteoclastogenesis and bone loss [7] may explain the interaction of HIV infection and bone mineralization. The lack of differences in the rate of osteopenia and osteoporosis found in HIV-infected patients irrespective of the treatment received has the limitation of the relatively small population included in the study. The difference observed in patients exposed to indinavir with respect to other patients treated in the femoral neck is a very preliminary observation; however, indinavir is the only PI known to alter the activity of osteoblast alkaline phosphatase *in vitro* [8].

The aetiology of bone loss in HIV-infected patients, the role of specific class toxicity, the clinical implications and the therapeutic or preventative strategies require further investigation.

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Received: 6 December 2000; accepted: 18 January 2001.

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Cidofovir added to highly active antiretroviral therapy in AIDS-associated progressive multifocal leukoencephalopathy

Progressive multifocal leukoencephalopathy (PML) is a demyelinating central nervous system disease occurring in HIV-infected as well as in other immunosuppressed patients. Its incidence has been reduced with the use of highly active antiretroviral therapy (HAART) [1] and some cases of cure after HAART have been described [2]. However, it continues to be a disabling disease with a high mortality rate.

De Luca *et al.* [3] reported that adding cidofovir to HAART results in a more effective control of JC virus, and an improved neurological outcome and survival in patients with AIDS-associated PML than when using HAART alone. We agree with the authors' conclusion about the importance of the early diagnosis and treatment of PML.

We have used cidofovir as a compassionate treatment for PML in eight patients with HIV infection, in addition to HAART. Written informed consent was obtained from every patient. The mean age was 37.5 years and seven patients were men. The risk factor for HIV infection was intravenous drug use in five, and sexual risk relationships in three. The mean CD4 cell count at diagnosis was 78×10^6/l (range 3–227×10^6/l) and plasma HIV-RNA levels were 78 943 copies/ml (range 122–522 000 copies/ml). Two patients were receiving HAART before the diagnosis of PML. The diagnosis of PML was based on clinical and radiological findings consistent with PML, the detection of JC virus DNA in the cerebrospinal fluid, or brain biopsy. PML was the first AIDS-defining illness in four patients. The mean duration of symptoms before diagnosis was 22 days (range 7–75 days), and they consisted of focal cerebellum symptoms (N = 6), cranial nerve paralysis (N = 5), hemiparesis (N = 3), and visual loss (N = 3). Magnetic resonance imaging revealed bilateral, non-enhancing, hyperintense lesions on T2-weighed images in all patients, without evidence of mass effect. In half of the patients the lesions were located supra- and infra-tentorially. At PML diagnosis, HAART was continued in two patients and started in the other six. Treatment with cidofovir was initiated simultaneously with the diagnosis of PML in two patients.

In six patients, cidofovir was added to HAART at a median of 42 days after the PML diagnosis (range 21–60 days) because of clinical deterioration. Cidofovir was administered at 5 mg/kg intravenously at 1 week intervals for the first two administrations, and every 2 weeks therafter, with probenecid and saline infusion. Five patients received two cidofovir cycles, one patient received four cycles and two, seven cycles. The low number of cidofovir cycles received by five of the patients was related to their short survival after beginning cidofovir (median 16 days). At follow-up, all patients except two showed clinical and radiological progression. Six patients (75%) died as a result of PML, at a median of 18 days after starting cidofovir (range 12–132 days). The other two remaining patients are still alive and their neurological symptoms, although present, have improved after a follow-up of 180 and 185 days, respectively.

Cidofovir is active against *Papovaviridae in vitro* [4], and recently its efficacy has been reported in the treatment of PML [3,5,6]. We agree with De Luca *et al.* [3] that patients with PML do not always benefit from HAART, and cases have occurred during HAART and despite a good virological response, as happened with two of our patients. Therefore, we think that an adjunctive therapy, such as cidofovir, may be useful for the treatment of PML. However, our findings suggest that cidofovir has a very limited effect in patients with PML and advanced HIV-related immunosuppression who are not showing a response to HAART, because the patients died before a significant number of doses of cidofovir could be administered. Considering that our experience is limited to a small number of patients, we think that cidofovir might be a therapeutic option for PML treatment, but should be prescribed in addition to HAART immediately after diagnosis.

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Received: 5 January 2001; accepted: 10 January 2001.

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A simple and rapid magnetic bead separation technique for the isolation of tetramer-positive virus-specific CD8 T cells

Recent reports on functional impairments within CD8 T cell subpopulations [1–5] focused attention on quantitative assessments of functional activity. However, the expansion and propagation of antigen-specific CD8 T cells often requires extensive tissue culture manipulations and frequent re-stimulations. A method that could selectively identify and expand an antigen-specific CD8 population would be of value in these quantitative assessments.

Flow cytometric or human leukocyte antigen (HLA) bead sorting of tetrameric-positive virus-specific T cells has proved to be of value [6,7]; however, it requires a flow cytometer, is technically demanding and needs conditions of absolute sterility. We have developed an assay that utilizes anti-phycoerythrin-coated MACS beads (Miltenyi Biotec, Auburn, USA) to select positively phycoerythrin-conjugated tetramer peptide complexes admixed with CD8 T cells to obtain and select enriched specific populations for in-vitro culture. This simplified method can lead to the rapid expansion of an antigen-specific T cell population.

To compare the isolation of virus-specific CD8 T cells by anti-phycoerythrin bead separation and flow cytometry, an HLA-A*0201-positive cytomegalovirus (CMV)-seropositive donor with 0.43%, CMV-pp65 (495–503: NLVPMVATV)-specific tetramer-positive CD8 T cells was identified (Fig. 1a). We isolated CMV-pp65 CD8 T cells with anti- phycoerythrin MACS beads by the incubation of 5×10^6 peripheral blood mononuclear cells (PBMC) for 20 min at 37°C with the HLA-A*0201 CMV-pp65-specific tetramer [8]. The PBMC were then incubated with 20 μl anti-phycoerythrin MACS beads (Miltenyi Biotec) at 4°C for 20 min to label the CMV-pp65-specific CD8 T cells. The labelled PBMC were passed through a pre-washed (500 μl of buffer: PBS pH 7.2, 0.5% bovine serum albumin and 2 mM ethylenediamine tetraacetic acid) separation column (Miltenyi Biotec) on a magnet. The column was eluted three times with 500 μl of buffer to remove the unbound CMV-pp65 tetramer-negative PBMC (negative fraction). The separation column containing the phycoerythrin-specific magnetic beads bound to the tetramer-positive T cells was removed from the magnet. The column was washed twice again with 500 μl of buffer then, finally, to ensure all the cells were recovered, a plunger was used to elute the labelled CMV-pp65 tetramer-positive CD8 T cells (positive fraction). Simultaneously, 5×10^6 PBMC were counterstained with a combination of phycoerythrin-conjugated CMV-pp65 tetramer and fluorescein-isothiocyanate-conjugated CD8 monoclonal antibody for flow cytometric isolation, performed using standard methods [9]. The CMV-pp65 tetramer-positive, negative and fluorescence-activated cell sorted (FACS) fractions were cultured in R15-50 medium [RPMI 1640 media supplemented with 15% heat-inactivated fetal calf serum, L-glutamine, hepes, 50 IU/ml IL-2 and penicillin–streptomycin (Biowhittaker, Maryland, USA)] with the addition of pooled irradiated PBMC feeders (at 1×10^6/ml) and anti-CD3 (12F6, Dr J. Wong, Massachusetts, USA).

After 14 days, the lytic activity of the CMV-pp65-specific CD8 T cell cultures was assessed in a standard ^{51}Cr release assay using CMV-pp65 peptide pulsed HLA-A*0201 B cell lymphoma targets. The CMV-pp65-specific CD8 T cells were added at two effector : target ratios and incubated at 37°C, 5% carbon dioxide for 4 h (Fig. 1c).

The CMV-pp65-specific CD8 T cell population was strongly CMV-pp65 tetramer positive after a 14 day in-vitro expansion period (Fig. 1b), whereas the 'negative' fraction remained CMV-pp65 tetramer negative (Fig. 1b). From a starting population of 5×10^6 PBMC, we initially isolated approximately 21.5×10^3 tetramer-positive cells (0.43%). This culture was expanded by stimulation with peptide-pulsed irradiated feeders over 14 days, with the result that 33.8% of the CD3/CD8 population were HLA-A*0201 CMV-pp65-specific tetramer positive (Fig. 1b). The ^{51}Cr release assay determined the specificity and function of the antigen-specific CD8 T cells against CMV pp65-peptide pulsed HLA-A*0201 targets. The percentage of specific target cell lysis of target cells was compared for anti-phycoerythrin MACS bead-enriched CMV pp65 antigen-specific CD8 T cell population and the corresponding expanded effector population, isolated by single-cell sorting using phycoerythrin-conjugated tetrameric complexes and FACS (Fig. 1c). The negatively sorted T cell population demonstrated non-significant lysis ($< 15\%$) compared with the positively sorted HLA-A*0201 CMV-pp65-specific CD8 population ($> 50\%$) (Fig. 1c). These data showed that MACS separation with anti-phycoerythrin micro beads is a viable technique for the separation of short-term cell lines and for the cloning of HLA tetrameric complex-labelled antigen-specific T cells.

Here we have demonstrated a simple immunomagnetic separation technique that utilizes technology readily available to most immunology laboratories worldwide. The method is specific and represents an inexpensive, reliable alternative to cell sorting by flow cytometry for the isolation of phycoerythrin-conjugated tetramer-positive T cells.

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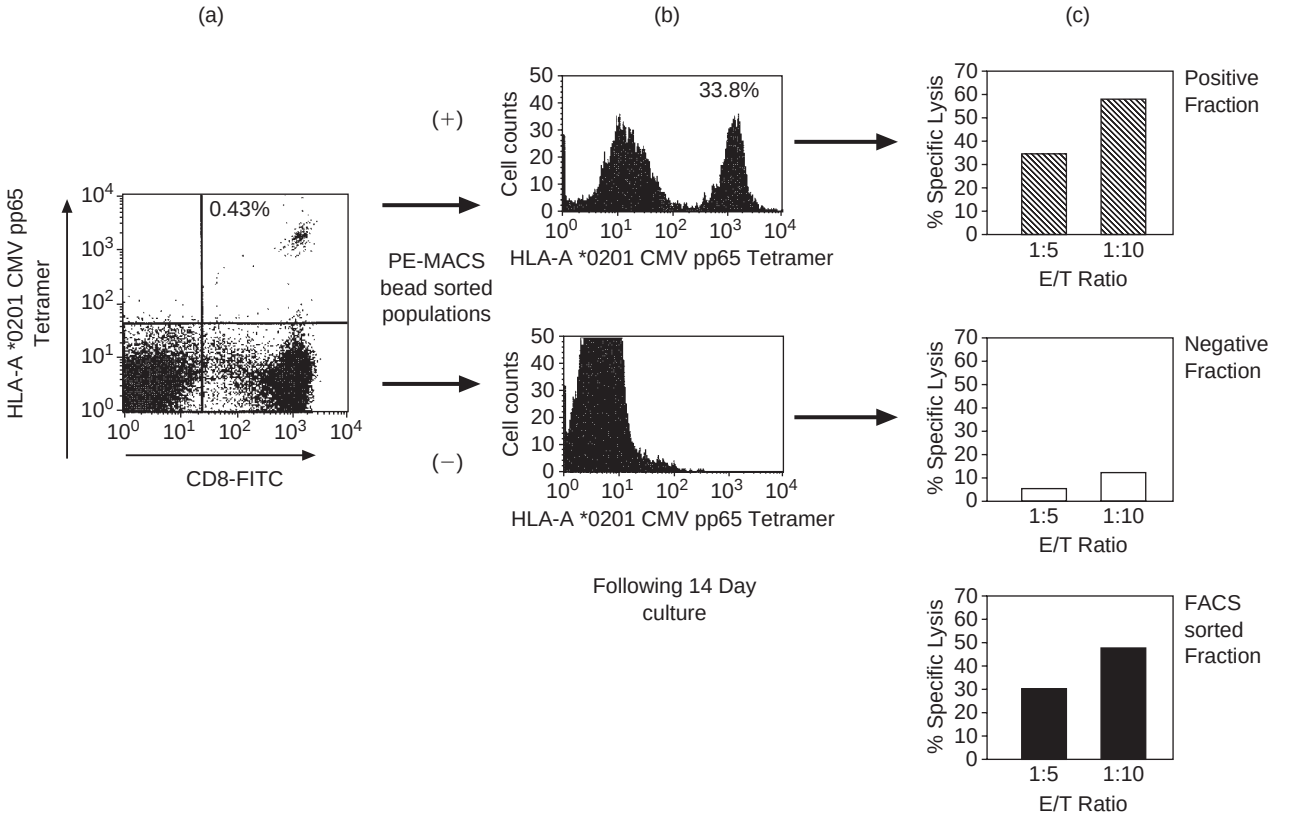

Fig. 1 (a) Dot plot of HLA-A*0201 CMV-pp65-specific CD8 tetramer staining gated on the CD3/CD8 population. From an HLA-A*0201 homozygous individual there were 0.43% tetramer-positive HLA-A*0201 CMV-pp65-specific CD8 T cells represented in the upper right quadrant. (b) Histograms representing the tetramer specificity of the anti-phycoerythrin-MACS bead for the isolation of human leukocyte antigen (HLA) tetrameric complex labelled antigen-specific T cells, after 14 days of stimulation with CMV-pp65 peptide and irradiated peripheral blood mononuclear cell (PBMC) feeders. The cultures were analysed for HLA-A*0201 CMV-pp65-specific CD8 T cells by flow cytometry. The lower panel represents the HLA-A*0201 CMV-pp65 tetramer positively selected population isolated by anti-phycoerythrin beads and the upper panel the 'negative' eluted fraction. The HLA-A*0201 CMV-pp65-specific CD8 T cells in the upper panel represent 33.8% of the CD3/CD8 tetramer-positive population. (c) Specific lysis of peptide pulsed B cell lymphoma target by HLA-A*0201 CMV-pp65 tetramer-specific CD8 T cells isolated by anti-phycoerythrin-MACS beads or by sorted using fluorescence-activated cell sorting (FACS) after 14 days of culture. The HLA-A*0201 CMV-pp65 tetramer-'positive' CD8 T cells and those cultured from the 'negative' fraction were assayed in a ^{51}Cr release assay at 1 : 5 or 1 : 10 effector to target (E : T) ratios. In addition, HLA-A*0201 CMV-pp65-specific CD8 T cells isolated by FACS were included, to compare the efficiency of the two methods.

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Sponsorship: This work was supported by NIH grant R01-AI-46254. Douglas F. Nixon is an Elizabeth Glaser Scientist of the Elizabeth Glaser Pediatric AIDS Foundation.

Received: 19 January 2001; accepted: 25 January 2001.

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The impact of experiencing lipodystrophy on the sexual behaviour and well-being among HIV-infected homosexual men

Of 117 HIV-infected homosexual recipients of highly active antiretroviral therapy (HAART), 50.4% reported lipodystrophy. Experiencing lipodystrophy had a strong impact on the perceived health and confidence in relationships of these men. An additional decrease in the enjoyment of sex and sexual activity was noted, although this may also be the result of other aspects of HAART use. The findings underline the importance that HIV-infected individuals, who are considering starting HAART, are informed about the possibility of such effects. Clinicians should be aware of the potential impact of HAART on their patients in case side-effects develop.

The use of HAART has been associated with changing views about high-risk sex [1,2]. In a previous study among homosexual men, not short-time HAART use (< 1 year) in itself, but the first HAART-induced virological and immunological improvements were likely to be associated with the (temporary) increased practice of risk behaviour [3]. Just as is observed for these specific favourable consequences of HAART, experiencing side-effects may also influence sexual behaviour in some way. Increasing attention is paid to lipodystrophy, a syndrome marked by the redistribution of body fat, and mostly attributed to the use of protease inhibitors (PI) [4–7]. Because this syndrome is associated with involuntary changes in body composition, we hypothesized that this may have a substantial influence on an individual's well-being and behaviour.

We sent a questionnaire to the HIV-positive homosexual men ($n = 176$) who participated in the Amsterdam Cohort Studies, and were now being seen by practitioners and clinicians in Amsterdam. The response rate was high: 141 men (80.1%) completed and returned the questionnaire. Of these, 117 (83.0%) received HAART, including PI for all individual but one. Of the 117 men, who were 42.3 years of age on average [standard deviation (SD) = 8.8], 40.9% ($n = 38$) had a college degree, and 89.7% ($n = 105$) were of northern or central European nationality. Information on the duration of HIV infection was available for 38 men, who were infected for on average 8.8 years (SD = 4.6). Information on the date of starting HAART was available for 99 men, who started HAART on average 2.8 years ago (SD = 0.6). We asked the men 'Have you ever experienced a change in fat distribution after initiating HAART (meaning: extremities getting thinner and abdominal size increasing, in medical terms called lipodystrophy)', and 50.4% ($n = 59$) reported lipodystrophy. In concordance with previous studies [4–7], men who reported lipodystrophy were older (mean age 45.4 years; SD = 8.3), had been HIV infected for a longer period (mean 10.6 years; SD = 4.1), and had started HAART earlier (on average 2.9 years ago; SD = 0.5) than men not reporting lipodystrophy, who on average aged 39.1 years (SD = 8.3), were HIV infected for 5.6 years (SD = 3.6), and had started HAART 2.7 years (SD = 0.6) ago. Whether or not information on the duration of infection or time since starting HAART was available was not related to reporting lipodystrophy, and neither was the level of education or nationality.

We asked the 59 men who reported lipodystrophy to compare the period of experiencing this syndrome with the period before. Comparisons were made regarding sexual behaviour and well-being (Table 1), using a five-point scale (1: much less; 2: less; 3: similar; 4: more; 5: much more). A *t*-test was used to determine whether the mean scores differed from the neutral score (score 3, indicating no change). The 59 men who experienced lipodystrophy reported a drastic decrease in sexual activity (Table 1). Importantly, they less enjoyed sex less, felt less physically well and were less confident in relationships when experiencing lipodystrophy. In analyses of variance, answers were not influenced by sociodemographic characteristics, time of being HIV positive or time since initiating HAART (all P values > 0.05).

This cross-sectional study was somewhat limited by the way in which variables were measured. Lipodystrophy was self-reported, which may have led to an under- or overestimation of the prevalence of 'clinical' lipodystrophy. Although some investigators used objective 'metabolic' criteria to define lipodystrophy, most other studies have also based their findings on subjective judgement of the syndrome [4–7]. Furthermore, in-

Table 1. Mean score on nine different items for which HIV-infected homosexual men who had ever experienced lipodystrophy as a result of highly active antiretroviral therapy (n = 59) were asked to compare the period in which they had experienced lipodystrophy with the period before, Amsterdam Cohort Study 2000.

	N = 59		
Items	Score (mean)	SD	*P* value[a]
Enjoying sex	2.40	(0.82)	< 0.001
Practice of sex	2.35	(0.81)	< 0.001
Practice of anal sex	2.38	(0.83)	< 0.001
Number of sexual partners	2.28	(0.86)	< 0.001
Condom use in anal sex with steady partners	3.00	(0.79)	1.000
Condom use in anal sex with casual partners	3.04	(0.73)	0.699
Physical health	2.51	(1.00)	< 0.001
Confidence in relationships	2.60	(1.08)	0.007

[a] *P* value indicates the level of statistical significance testing whether the mean score differs from the neutral score (score 3: no difference).

dividuals were asked to recall events afterwards. Recall bias might be introduced depending on the duration and severity of lipodystrophy, as well as the presence of this syndrome at the time of measurement. To what extent recall bias influenced our results, however, is unknown.

In discussing the specific impact of experiencing lipodystrophy, one should also take into account changes that occurred as a result of the use of HAART *per se*. Therefore, we also asked men to compare the entire period of using HAART with the period before (in which they knew they were HIV positive) (data not shown). Over the entire HAART period, no change was reported in self-perceived health and confidence in relationships, contrasting with the strong decrease over the period of experiencing lipodystrophy. Therefore, lipodystrophy has probably a substantial and specific impact on a person's well-being, which can not be attributed to the use of HAART in general. Men who ever experienced lipodystrophy reported a decrease in sexual activity and the enjoyment of sex over the entire HAART period, just as over the specific period of experiencing this syndrome. This may indicate that lipodystrophy has a very strong impact on these changes, because these changes are still reflected when asked over the entire period of HAART use. On the other hand, a decrease in sexual activity in the period of using HAART was also reported by men who never experienced lipodystrophy, indicating a role of other HAART-related factors, such as the possible sexual dysfunction associated with PI use [8,9]. Over the period of experiencing lipodystrophy no change was reported in condom use, indicating that a direct impact of lipodystrophy on condom use is not likely. However, men who experienced lipodystrophy did increase their condom use when asked over the entire HAART period (whereas men not reporting lipodystrophy did not). This finding probably results from other HAART-related differences between the two groups, although differences in the time since starting HAART or age appeared not to play a role.

In conclusion, a large proportion of HAART recipients experienced lipodystrophy, which probably had a strong impact on the perceived health and confidence in relationships of these men. An additional decrease in enjoying sex and sexual activity has been noted, although we cannot determine whether this is caused by lipodystrophy, other aspects of HAART, or both. The findings underline the importance of fully informing HIV-infected individuals who are considering starting HAART about the possibility of side-effects. For clinicians, this study indicates how their patients may react to HAART or HAART-related side-effects, in particular lipodystrophy, and prepares them to assist their patients in coping with such effects.

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Received: 25 October 2000; revised: 2 February 2001; accepted: 6 February 2001.

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Syphilis and gonorrhoea in Paris: the return

We report here on 10 cases of early syphilis in men diagnosed in our sexually transmited diseases (STD) centre in Paris between March and November 2000. We also report a 104% overall increase of bacteriological confirmed gonococcal infections (BCGI) during the same 9 month study period during 5 years.

It has been reported that an increase in sexual risk-taking among San Fransisco gay men has correlated with increasing rates of rectal gonorrhoea in this population [1]. Recently, an increase in the incidence of gonorrhoea in London and in other places in the UK has been reported [2,3]. In 1999, an epidemiological study in France (the RENAGO study) [4] suggested an increase in the incidence of gonococcal infections between 1997 and 1998. A limited syphilis outbreak has also been reported in the USA [5], but no information on new cases of early syphilis is available from France.

Between March 2000 and November 2000, one heterosexual man and nine homosexual men consulted at our STD centre for early syphilis. Five patients were HIV positive, three were HIV negative, and the HIV status was unknown in the last two cases because the patients declined a blood test for HIV serology. The mean clinical and biological features of the patients are summarized in Table 1. Three patients were diagnosed with primary syphilis and seven were diagnosed with secondary syphilis. One HIV-positive patient had previously been hospitalized in 1994 for neurosyphilis, another HIV-positive patient had urethritis 2 weeks before consulting for secondary syphilis, and a third HIV-infected patient had concomitant non-specific urethritis. Two HIV-infected patients were undergoing highly active antiretroviral therapy (HAART) combining stavudine, lamivudine and indinavir, with respective CD4 cell counts of 667 and 250/mm^3, the HIV-RNA plasma viral load was 17 000 copies per ml in one patient and undetectable in the other. The three other HIV-infected patients were not treated with antiviral therapy, their CD4 cell count were 829, 601, and 260/mm^3, respectively, and their HIV-RNA plasma viral loads were 4490 and 427 copies/ml and not available in the last case. None of the HIV-infected patients had neurosyphilis. Patients were treated with 2.4×10^6 units of benzathine penicillin G.

These observations suggest a large increase in high-risk sexual practices among gay men living in Paris, because during the past few years no new cases of early syphilis had been diagnosed at our STD center. This is in accordance with a large increase in gonoccocal infections reported in our center since 1995. In Table 2, we report the number of BCGI and the total of STD leading to treatment for gonococcal infection, comprising BCGI and urethritis or rectitis suspected of gonococcal origin because of both clinical symptoms and

Table 1. Mean clinical and biological features of the 10 gay men presenting with early syphilis.

				Serology of syphilis		
Patient	Age (years)	HIV serology	Stage of syphilis	VDRL (units)	TPHA (titre)	FTA (titre)
1	38	+ CD4 cells: 667/mm^3 VL: 17 000 copies/ml	Secondary	128	1/10 240	1/640
				Positive dark-field on cutaneous lesion		
2	42	+ CD4 cells: 824/mm^3 VL: 4490 copies/ml	Secondary	8	1/640	1/800
3	29	+ CD4 cells: 601/mm^3 VL: 427 copies/ml	Secondary	32	1/2560	NA
4	42	+ CD4 cells: 250/mm^3 VL: not detectable	Secondary	16	1/20 480	1/1600
5	25	+ CD4 cells: 260/mm^3 VL: NA	Secondary	64	1/640	NA
6	64	Negative	Secondary	4	1/20 480	1/3200
7	34	Negative	Primary	4	1/1280	1/200
8	40	Unknown	Primary	16	1/1280	1/800
9	41	Unknown	Primary	2	±	±
				Positive dark-field result		
10	41	Negative	Secondary	256	> 1/20 480	NA
				Positive dark-field on cutaneous lesion		

FTA, Fluorescent treponemal antibody; TPHA, *Treponema pallidum* haemagglutination antibody; VDRL, Venereal Disease Research Laboratory; VL, viral load.

Table 2. Number of gonococcal infections during 5 years

Year	BCGI	Total of patients treated for gonococcal infections	Visits to the STD centre (first 9 months) (no. of patients)
1996	23[a]	24	4753
1997	34	48	6333
1998	35	69	6476
1999	43	72	5940
2000	47[a]	86	5044

[a]There was a 104% overall increase in bacteriological confirmed gonococcal infections (BCGI) between 1996 and 2000 (for the first 9 months).
STD, Sexually transmitted diseases.

presence of intracellular Gram-negative diplococcus after Gram staining, but which cannot be isolated after culture (total gonococcal infections). As is shown, we observed a 104% overall increase in BCGI, with 23 cases in 1996 and 47 cases in 2000. As is reported, there was also an increase in the proportion of suspected versus BCGI cases since 1997, corresponding with the introduction of HAART for the treatment of HIV-infected patients. It could be that HAART may interact with the culture capacity of *Neisseiria gonorrhoeae*. This hypothesis needs to be explored further.

The increase in both early syphilis and gonococcal infections contrasts with the decrease in the number of visits to our STD centre, with 6476 consultations for the first 9 months of 1998 falling to 5044 consultations during the same period in 2000 (Table 2). We therefore emphasize the need for continued surveillance of these STD in Paris and in other places in France, in order to focus sexual health promotion activities on controling their increasing incidence and preventing the risk of new HIV contaminations.

Acknowledgements

The authors would like to thank the staff of the STD Center at Hôpital Tarnier, Paris, France.

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Sponsorship: Nicolas Dupin is the recipient of a grant from SIDACTION.

Received: 15 December 2000; accepted: 18 January 2001.

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Partner notification by HIV-1 seropositive pregnant women: association with infant feeding decisions

Each year, an estimated 600 000 perinatal HIV-1 infections occur despite the availability of interventions to decrease mother-to-child transmission of HIV-1 [1]. In order to implement perinatal HIV-1 interventions it is necessary to diagnose HIV-1 infection during pregnancy. Even with antenatal HIV-1 counselling and testing, however, pregnant HIV-1 seropositive women in developing countries are often unable to access antiretroviral agents or avoid breastfeeding because of financial constraints and the fear of stigmatization.

Pregnant HIV-1 infected women may choose to notify their spouses/partners of their HIV-1 status in order to gain emotional and financial support. The majority of women, however, do not inform their partners, and encouraging women to notify spouses/partners that they are HIV-1 seropositive remains controversial [2]. Studies among non-pregnant HIV-1 seropositive women found that less than half of the individuals notified their sexual partners when they learned they were HIV-1 infected [3,4]. A study among pregnant women found that only 27% chose to notify their spouses/partners about their positive HIV-1 serostatus [5]. The fear of domestic violence or abandonment is frequently cited as a reason why not all women inform their spouse/partner about their HIV-1 status [6–8].

Voluntary HIV-1 counselling and testing in the antenatal setting provides unique incentives for women to inform spouses/partners about their HIV-1 status and include them in efforts to prevent HIV-1 infection in their unborn child. A better understanding of issues surrounding partner notification in the antenatal setting may lead to the improved uptake of interventions to reduce mother-to-child HIV-1 transmission.

From August 1999 to March 2000, pregnant women were screened for HIV-1 in five Nairobi City Council antenatal clinics. HIV-1 seropositive women were referred to Kenyatta National Hospital for further counselling and recruitment into a perinatal HIV-1 cohort. Women were enrolled after completing at least two post-test HIV-1 counselling sessions with health-care professionals and peer counsellors. Enrolled women completed a questionnaire providing detailed demographic data, information about partner notification, and infant feeding decisions. All women were provided with short-course antiretroviral therapy to reduce the risk of infant HIV-1 infection. Statistical analysis was performed using Pearson's chi-square test, Fisher's exact test, and logistic regression for multivariate analysis.

Among 7580 women offered HIV-1 testing, 7071 (93%) accepted testing, 1059 (15%) were HIV-1 seropositive, and 883 (83%) HIV-1 seropositive women returned for test results. Of the 172 (19%) HIV-1 seropositive women enrolled in the cohort study, 116 (67%) reported informing their partners about their HIV-1 status and 41 (25%) brought their partners for HIV-1 counselling. Reported partner notification was significantly associated with non-polygamous marriage, the absence of a personal income, living in a single room, and no previous history of a sexually transmitted disease (STD) (Table 1). There was no association with education level, marriage duration, or parity.

Women who informed their partners about their HIV-1 seropositive status were significantly more likely to choose formula feeding over breastfeeding than women who did not inform their partners [21% versus 9%; odds ratio (OR) 2.7; 95% confidence interval (CI) 1.0–7.5]. In a multivariate analysis controlling for marital status, employment, living in a single room, and previous STD history, partner notification was significantly associated with the decision to formula feed (OR 3.6; 95% CI 1.1–11.6).

In this study, several socioeconomic indicators were identified as correlates of partner notification among pregnant women. Women without financial independence and women living in a single room were more likely to inform their partners that they were HIV-1 seropositive. These women may perceive a need for economic support from their spouse/partner in the setting of HIV-1 seropositivity. Alternatively, they may be less cognizant of the risks associated with partner notification.

An important association between partner notification and a woman's decision to formula feed her infant was also found. In societies in which breastfeeding is the norm, it may be difficult for women to decide to use formula without spousal consent. In addition, because formula is costly, it may not be feasible for a woman to choose to formula feed without her partner's economic support. Although we provided zidovudine to all women in this study, in a non-research setting the use of antiretroviral agents may also require the economic support of the spouse/partner. For some women partner notification of HIV-1 status may thus be essential to obtain access to interventions to prevent mother-to-child HIV-1 transmission.

Determining the most appropriate way to incorporate spouses/partners into efforts to decrease mother-to-child transmission of HIV-1 will be challenging, particularly when women are in abusive relationships or when couples are HIV-1 serodiscordant. More research is necessary to explore barriers to partner notification. In addition, methods for safely increasing partner notification need to be investigated to determine their acceptability, feasibility, and effect on the uptake of interventions to prevent mother-to-child HIV-1 transmission.

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Table 1. Correlates of partner notification.

Correlate	Number notifying partner (%)	Number not notifying partner (%)	Odds ratio	95% CI
Non-polygamous marriage	105 (78)	30 (22)	8.2	3.7–18.7
No personal income	88 (72)	34 (28)	2.0	1.0–4.0
Living in single room	101 (71)	41 (29)	2.5	1.1–5.6
No previous STD history	92 (72)	35 (28)	2.3	1.1–4.6
Age < 24 years	68 (74)	24 (26)	1.7	0.9–3.4
Education < 8 years	34 (68)	16 (32)	1.0	0.5–2.0
Married ⩾ 5 years	14 (29)	35 (71)	1.3	0.6–2.7
Nulliparous	11 (27)	30 (73)	0.7	0.3–1.5

CI, Confidence interval; STD, sexually transmitted diseases.

Sponsorship: This work was supported by the US National Institutes of Health through grant R01 HD-23412. C. Farquhar was a scholar in the International AIDS Research and Training Program supported by the Fogarty International Center (T22-TW00001).

Received: 1 November 2000; revised: 1 February 2001; accepted: 6 February 2001.

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