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Plasma levels of B-lymphocyte stimulator increase with HIV disease progression

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We measured the plasma levels of B-lymphocyte stimulator (BLyS) in 101 HIV-1-infected patients and 18 controls. BLyS levels were higher among HIV-positive patients [median 5.70 (3.90) versus 4.62 (1.04) ng/ml, P = 0.002], who had significantly higher BLyS and total serum globulin levels with decreasing CD4 cell counts. Moreover, BLyS levels increased exponentially below 100 CD4 cells/μl. BLyS and globulin levels increase as HIV disease progresses, suggesting a role for BLyS in the hypergammaglobulinemia of HIV infection.

HIV infection is often accompanied by polyclonal hypergammaglobulinemia [1–5], resulting from a state of generalized, non-specific B-cell activation [6–8]. The mechanism underlying this phenomenon has not been conclusively established. B-lymphocyte stimulator protein (BLyS) is a member of the tumor necrosis factor ligand superfamily that regulates the survival, proliferation and differentiation of B lymphocytes [9–13]. *In vitro*, BLyS induces B-cell activation and expansion [12,14]; *in vivo*, exogenous administration of BLyS to mice leads to the expansion of B-cell populations in lymphoid tissue and increased serum immunoglobulin levels [11,12]. In humans, BLyS levels are elevated in autoimmune disorders associated with hypergammaglobulinemia [15–18] and in follicular non-Hodgkin's lymphoma [17]. Given the similarities between the biological actions of BLyS and the humoral immune derangements seen in HIV infection, we hypothesized that BLyS levels may be abnormal in HIV disease.

A total of 101 adult HIV-infected patients and 18 healthy, HIV-uninfected volunteers were included in the study. Demographic data, nadir CD4 T-cell counts, highest plasma HIV-RNA levels, history of antiretroviral treatment, and the CD4 T-cell count, plasma HIV-RNA level and serum globulin level values closest in time to the measurement of BLyS were recorded for HIV-infected patients. HIV-infected patients were subdivided into three groups according to their most recent CD4 T-cell count: group 1, greater than 500 cells/μl (n = 27); group 2, 201–500 cells/μl (n = 42) and group 3, 200 cells/μl or less (n = 32). BLyS levels were measured by enzyme-linked immunosorbent assay on stored plasma samples as previously described [16].

BLyS levels were significantly higher among HIV-infected patients than among controls [median (interquartile range; IQR), 5.70 (3.90) versus 4.62 (1.04) ng/ml, $P = 0.002$]. Ninety per cent of the controls had BLyS levels between 3.4 and 5.6 ng/ml. None of the controls had a BLyS level above 5.7 ng/ml, in agreement with previous reports, which have generally found BLyS levels below 10 ng/ml in normal individuals [15–17]. By comparison, 51 of the HIV-infected patients (50.5%) had levels above 5.7 ng/ml and 22 of them (21.8%) had levels above 10 ng/ml. BLyS levels in the subgroups of HIV-1-infected patients and the controls are depicted in Fig. 1a. There was a graded trend towards increasing BLyS levels with more advanced stages of HIV disease. Median (IQR) BLyS levels among uninfected controls and HIV-infected patients with over 500, 201–500 and fewer than 200 CD4 T cells/μl were 4.62 (1.04), 4.85 (2.97), 5.51 (2.20) and 8.28 (7.43) ng/ml, respectively ($P < 0.001$). The corresponding serum globulin levels among HIV-infected patients were 3.3 (0.4), 3.6 (1) and 3.8 (6.8) g/dl ($P = 0.026$), paralleling the trend observed in BLyS levels (Fig. 1b); however, the linear correlation between the two did not reach statistical significance. There was a significant but non-linear correlation between BLyS levels and CD4 T-cell counts, with BLyS levels remaining relatively stable at higher CD4 T-cell counts and increasing exponentially at the lower extreme of the CD4 T-cell count spectrum. There was a weak, but statistically significant direct linear correlation between plasma HIV-RNA and BLyS levels.

The elevation of BLyS levels in our HIV-infected patients is consistent with findings in a previous report on the production of autoantibodies at different stages of HIV infection [19]. Although both plasma BLyS and serum globulin levels proved to be strongly associated with the stage of HIV disease in our cohort, we were unable to confirm a direct correlation between them. This does not exclude the possibility that an underlying correlation between BLyS and immunoglobulin levels might have been obscured in this analysis by other serum globulins or by the effect of regulatory signals other than BLyS on immunoglobulin production. In a previous report on a similar cohort of HIV-infected

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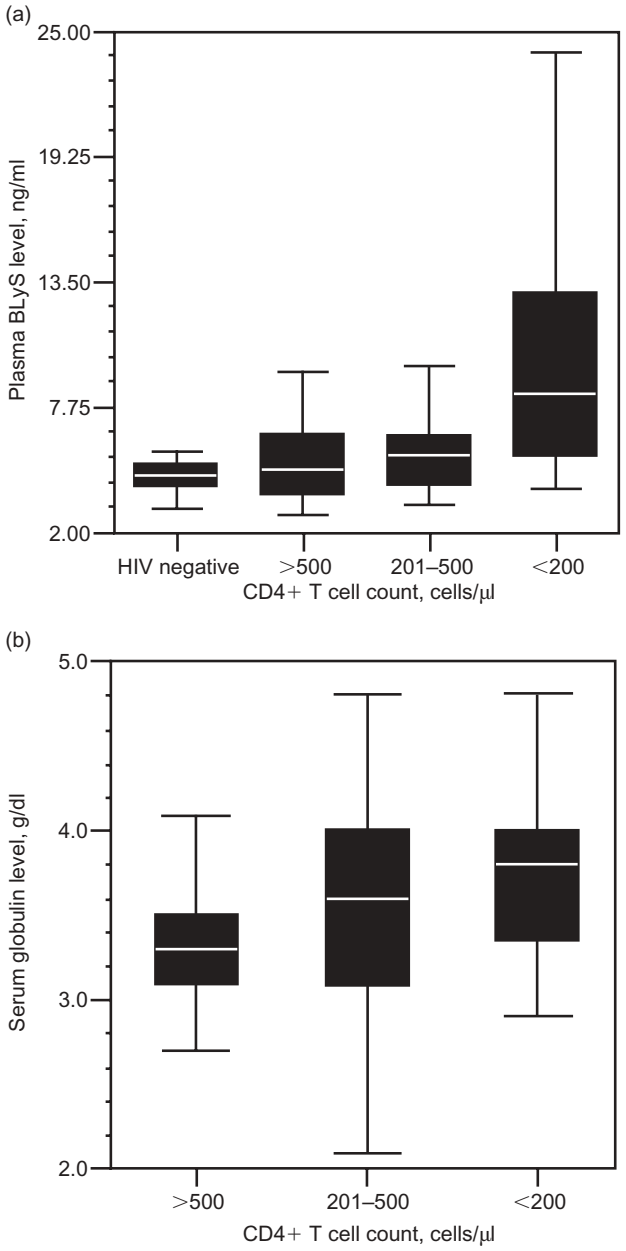

Fig. 1. B-lymphocyte stimulator and serum globulin levels according to HIV infection stage. The white lines represent the median, the boxes represent the interquartile range, and the bars represent the range of the respective markers. (a) B-lymphocyte stimulator (BLyS) levels are increased with more advanced HIV-induced immune deficiency. BLyS levels in normal volunteers are also included for comparison. Note the graded increase in BLyS levels with more advanced stages of HIV disease, as reflected by lower CD4 T-cell counts. (b) Serum globulins are increased with more advanced HIV-induced immune deficiency. Note similarity with the trend observed in BLyS levels.

patients [19], no significant association could be demonstrated between BLyS and IgG levels regardless of the CD4 T-cell count. In patients with autoimmune disorders, a weak association between BLyS and immunoglobulin levels has been found by some investigators [15], but not others [18].

This study does not allow us to determine the mechanism of BLyS level elevation in HIV infection. The cells that primarily express BLyS, including dendritic cells, monocytes and macrophages [9,10,12,20], are all targets for HIV, suggesting that HIV might directly drive the overexpression of BLyS by these cells. Alternatively, the expression of BLyS may be upregulated as a homeostatic response to lymphopenia or by soluble factors that are, in turn, elevated in HIV infection. Both IFN-γ and IL-10 upregulate BLyS expression and secretion [12,21]. Because its production is impaired in the more advanced stages of HIV disease [22], IFN-γ is an unlikely mediator of the increased levels of BLyS seen in HIV-infected patients. IL-10 levels, on the other hand, are often elevated in HIV infection [23–25], and are inversely correlated with CD4 T-cell counts [26]. Moreover, HIV Nef protein directly induces IL-10 expression and production *in vitro* [25], and effective antiretroviral treatment leads to a rapid decline in IL-10 levels [23]. One could thus speculate that in HIV infection, increased IL-10 production might result in the upregulation of BLyS with subsequent B-cell activation and hypergammaglobulinemia.

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Altered lymphocyte heat shock protein 70 expression in patients with HIV disease

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Heat shock protein (HSP) expression in lymphocytes isolated from 20 patients with HIV disease and 15 age-matched controls was determined. Fold increases in lymphocyte hsp70 expression after heat shock were 4.52 ± 2.97 in HIV-positive individuals compared with 2.60 ± 1.29 for HIV-negative controls ($P = 0.001$). Given clear roles for HSP in the cross-presentation of antigens, α-defensin internalization and pro-inflammatory cytokine production, a further investigation of HSP in HIV patients is merited.

Heat shock proteins (HSP) are highly evolutionary conserved proteins found in all organisms from bacteria to humans. HSP synthesis is also induced by cellular stressors other than heat, including heavy metal exposure, oxidative stress, viral and bacterial infection and thus the more general term 'stress protein' has been applied to this class of protein [1]. HSP perform essential functions in the cell associated with protein folding and assembly and the prevention of protein aggregation and degradation. Under normal conditions they act as molecular chaperones in ensuring that newly formed polypeptides are correctly transported to appropriate cellular organelles. They also act as cytokines and induce proinflammatory cytokine production in human monocytes [2].

The role of HSP in HIV disease pathogenesis is only beginning to be appreciated. A very recent report that the expression of the HSP receptor, CD91, is increased in monocytes from patients with long-term non-progressive HIV disease [3] is of particular importance given that key components of the soluble factor, termed CAF, which suppresses HIV replication, and which is secreted from stimulated CD8 T lymphocytes in high amounts from such individuals have been identified as α-defensins [4]. The latter have in turn been demonstrated to be associated with CD91, which mediates the internalization of α-defensins [5]. Furthermore, HSP are selectively incorporated into the HIV virion during the assembly process [6].

To date, HSP expression in patients with HIV disease has not been reported. Early studies have demonstrated the upregulation of hsp27 and hsp70 messenger RNA transcription in CD4 T-cell lines infected with HIV [7]. In this report, we demonstrated that the expression of lymphocyte hsp70 is altered in patients with HIV disease compared with uninfected age-matched controls.

Twenty patients (male, mean age 47 years) with HIV disease and 15 age-matched controls (male, mean age 45 years) were recruited. Fourteen of the HIV-positive cohort were currently receiving combination antiretroviral therapy and 13 had an undetectable viral load. The mean CD4 T cell count was 500 cells/mm^3 (SD $\pm$ 304).

Lymphocytes from 20 ml of venous blood were isolated using Ficoll-Paque gradient centrifugation and were incubated at either 37°C for 1 h (control) or heat shocked at 42.5°C for 1 h. Lymphocytes were then allowed to recover at 37°C for 3 h and proteins were extracted and separated by sodium dodecyl sulphate-polyacrylamide gel electrophoresis. Hsp70 expression was measured as previously described [8] using both Western immunoblots (β-actin used as an internal control) and enzyme-linked immunosorbent assay (StressGen Biotechnologies, Victoria, Canada). The

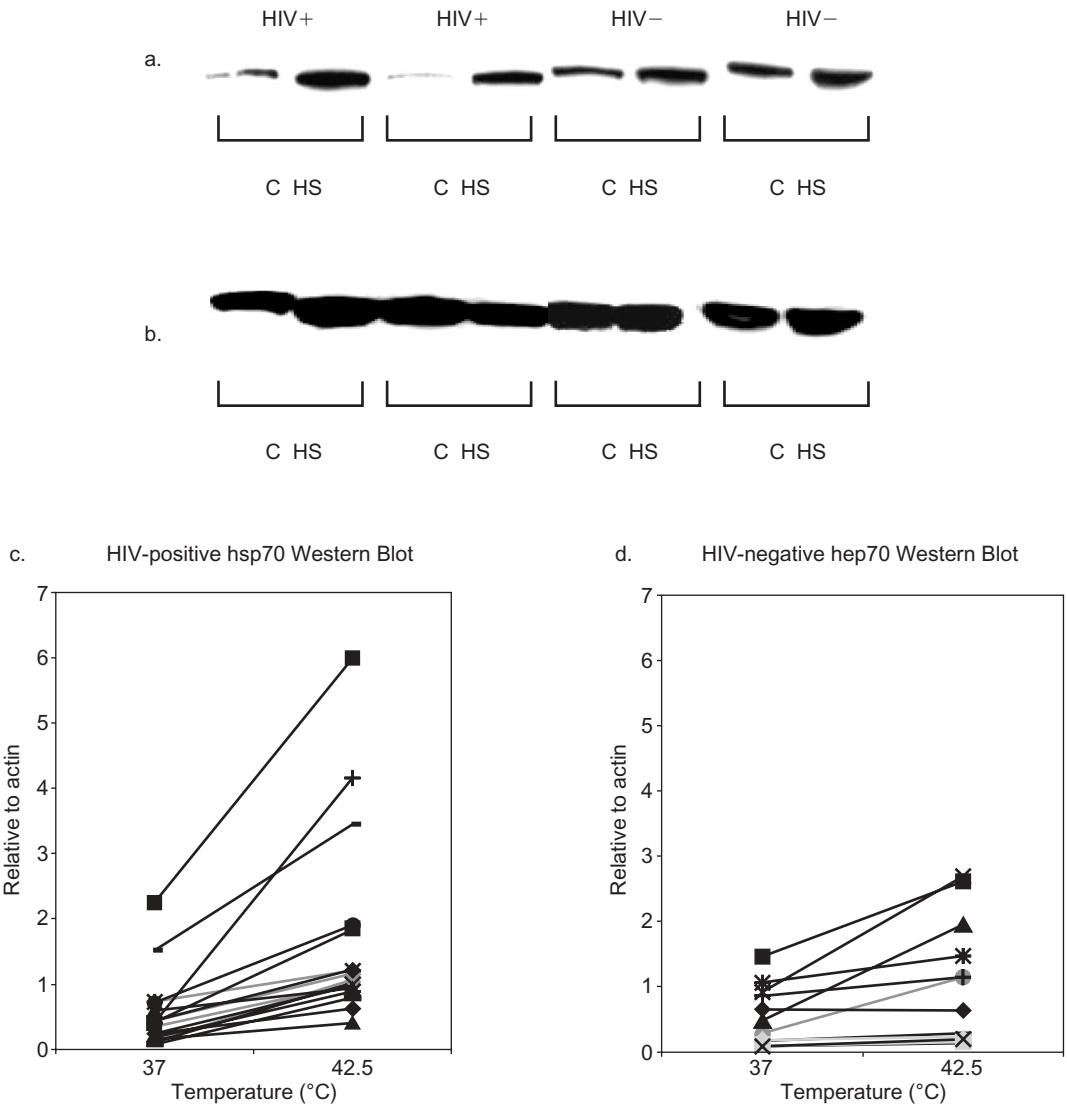

Fig. 1. Western blots and fold-increase of hsp70 in lymphocytes from HIV-positive and HIV-negative individuals. Lymphocyte hsp70 expression after heat shock (HS) is increased in patients with HIV disease relative to baseline values (C). (a) Representative samples for HIV-positive subjects and HIV-negative controls. Each patient is represented by one bracket. (b) Corresponding β-actin internal controls. (c) Fold increase in hsp70 (Western blot) is significantly increased in HIV-positive patients, as compared with HIV-negative controls (d).

fold increase in lymphocyte hsp70 expression was calculated by densitometric analysis and was expressed as the ratio of hsp70 expression at 42.5°C to that at 37°C. Correlations were sought between hsp70 expression and CD4 cell count, plasma viral load and antiretroviral treatment status. All procedures were approved by the Human Research Ethics Committee, University of New England (HEO 1/216) and the Research Ethics Committee-Eastern Section, Sydney (01/198).

Data were analysed using analysis of variance (general linear model) with control (non-heat shock) versus heat shock as the within-subject factor and HIV-positive versus HIV-negative as the between-subject factor.

Lymphocyte hsp70 expression after heat shock relative to baseline values was increased in both control and in patients with HIV disease. Significant augmentation in lymphocyte hsp70 expression after heat shock was demonstrated by Western blots. Representative Western immunoblots from HIV-positive and HIV-negative samples, together with corresponding β-actin controls, are presented in Fig. 1a,b. Fold increases in lymphocyte hsp70 expression (Fig. 1c,d) after heat shock were 4.52 ± 2.97 in HIV-positive individuals compared with 2.60 ± 1.29 for HIV-negative controls ($P = 0.001$). The increase in hsp70 expression in lymphocytes from HIV-positive individuals was primarily caused by the lower baseline level, rather than absolute amounts. Similar trends were demonstrated using enzyme-linked immunosorbent assay (results not shown). Using this assay, fold increases in lymphocyte hsp70 expression after heat shock were 4.88 ± 3.78 in HIV-positive individuals compared with 2.80 ± 1.21 in HIV-negative controls ($P = 0.065$).

In this relatively small cohort, no correlation between fold increases in lymphocyte hsp70 expression and viral load, CD4 T cell count or antiretroviral treatment status could be determined. Larger studies will be required to examine such associations.

The determinants of altered hsp70 expression in patients with HIV have not been analysed. Alterations in viral replication or oxidative stress may influence hsp70 expression. Increases in oxidative stress, a characteristic of HIV-positive infection, may alter HSP expression. In this respect, the modulation of HSP expression by oxidative stress and antioxidants [9] has been reported. Although no correlation between lymphocyte hsp70 expression and viral load was demonstrated in this study, we did observe a direct correlation between lymphocyte hsp70 expression and measures of oxidative stress. In the present study, we have demonstrated increased plasma protein carbonyl formation ($P < 0.05$) and decreased plasma antioxidant status ($P < 0.05$), both measures of oxidative stress, in this HIV-infected cohort (data not shown), which correlated with fold increases in lymphocyte hsp70 expression. Further studies are required to define these interactions.

This is the first report of altered hsp70 expression in patients with HIV disease. Lymphocyte hsp70 expression in response to heat shock is significantly increased relative to baseline values in patients with HIV disease. The significance of altered lymphocyte HSP expression remains to be determined. However, given the clear roles of these proteins in the cross-presentation of antigens, α-defensin internalization and pro-inflammatory cytokine production, further investigation is merited.

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Drug-induced aminotransferase alterations during antiretroviral HIV post-exposure prophylaxis

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In 655 individuals receiving HIV postexposure prophylaxis (PEP), drug-induced aminotransferase alterations were frequent and severe in the nevirapine-including regimen, rare and mild-to-moderate in other combinations, and always reversible. Grade 3–4 incidence in protease inhibitor or nevirapine PEP was 0.5 and 25.0 per 100 person-months, respectively. Apart from nevirapine, continuing PEP appears to be safe even in the case of aminotransferase alterations. The usefulness of routine monitoring of liver function during PEP could be re-considered.

Antiretroviral postexposure prophylaxis (PEP) is widely used after exposures to HIV to reduce the risk of transmission in the healthcare setting, and it has also been proposed for non-occupational exposures [1,2]. This large use of prophylaxis raises concerns about its safety.

Adverse events of antiretroviral drugs are frequent reasons for the discontinuation or modification of therapy [3,4], as well as of PEP [4,5]. In particular, hepatotoxicity can occur with any antiretroviral regimen [6], and it can complicate a hepatic co-morbidity. Furthermore, liver enzyme abnormalities have been described in 1–10% of individuals taking PEP [4,7,8].

To evaluate the features of hepatotoxicity during PEP in real practice, data collected from August 1996 to September 2002 in the Italian Registry of Antiretroviral Post-Exposure Prophylaxis [4] were reviewed.

Subjects were divided into three groups according to their initial PEP regimen: two nucleoside reverse transcriptase inhibitors (NRTI) (group A), two NRTI plus one protease inhibitor (PI) (group B), or one non-nucleoside reverse transcriptase inhibitor-containing regimen (group C). Only individuals who had taken PEP for at least 5 days, and for whom at least two values of plasma level of alanine aminotransferase (ALT) and aspartate aminotrasferase (AST) were available (the first one at baseline before the initiation of PEP and the second within 5 days from discontinuation of PEP), were included in the analysis. Hepatitis C virus (HCV), hepatitis B virus (HBV), and HIV serology were performed at the time of exposure and at the 6 month follow-up. AST and ALT changes from the baseline to the highest values were categorized according to the toxicity grading used by the AIDS Clinical Trials Group, modified by Sulkowski *et al*. [9]. If the grades of AST and ALT in the same determination were different, the highest one was considered.

Of the 1721 reports of PEP analysed, 207 individuals in group A, 429 in group B, and 19 in group C were eligible for the study, as described in Table 1.

Overall, 529 individuals took PEP for at least 28 days; antiretroviral agents were used at standard doses. The median duration of PEP was 30 days in groups A and B (range 5–60, and 5–57, respectively), and 25 days (range 5–32) in group C.

All subjects were tested for HIV, hepatitis B serum antigen (HBsAg), and HCV at the 6-month follow up. One case of acute hepatitis C with documented seroconversion was observed and was excluded from the following analysis.

In group A, six cases of grade 1 AST/ALT alteration occurred within 10–15 days; all the subjects completed their PEP without further increases in the AST/ALT levels.

In group B, grade 3 AST/ALT alteration was observed in two individuals taking zidovudine–lamivudine plus nelfinavir or indinavir, after 20 days, for an incidence rate of 0.5 per 100 person-months of PEP. Grade 2 AST/ALT alteration developed in six subjects between 10 and 30 days; and grade 1 in eight cases between 10 and 20 days. Four individuals discontinued PEP, two with grade 1 (one during a hypersensitivity reaction with generalized maculopapular rash after 7 days of treatment), two with grade 2 and grade 3, respectively. In addition, two subjects modified the initial PI-containing regimen because of grade 2 AST/ALT alteration, but were able to complete the 4-week PEP with two NRTI.

In group C, seven cases started PEP with an efavirenz-containing regimen; none developed AST/ALT alteration. Of the 12 subjects who took nevirapine, two cases of grade 4 AST/ALT alteration were observed after 11 and 28 days, respectively, for an incidence rate of 25 per 100 person-months. One subject required hospital admission. Both recovered promptly after PEP

Table 1. Baseline characteristics and antiretroviral drugs prescribed in 655 subjects receiving HIV post-exposure prophylaxis – Italian Registry of Antiretroviral Post-Exposure Prophylaxis, 1996–2002.

	Group A (n = 207)	Group B (n = 429)	Group C (n = 19)
Sex, female, n (%)	114 (55)	335 (78)	13 (68)
Age, year, mean (range)	35 (3–67)	35 (17–68)	36 (22–55)
Type of exposure, n (%)			
Occupational			
Healthcare workers	135 (65)	323 (75)	12 (63)
Non-healthcare workers	17 (8)	19 (4)	1 (5)
Non-occupational			
Sexual	20 (10)	43 (10)	2 (11)
Parenteral	35 (17)	44 (10)	4 (21)
HCV antibodies[a] n (%)			
Positive	6 (6)	6 (3)	–
Negative	90 (94)	188 (97)	14 (100)
HBsAg[b] n (%)			
Positive	–	3 (2)	–
Negative	64 (100)	124 (98)	12 (100)
Antiretroviral drugs, n (%)			
Zidovudine–lamivudine	189 (91)	390 (91)	16 (84)
Other two NRTI combinations	18 (9)	39 (9)	3 (16)
Indinavir	–	361 (84)	–
Nelfinavir	–	59 (14)	–
Other protease inhibitors	–	9 (2)	–
Nevirapine	–	–	12 (63)
Efavirenz	–	–	7 (37)

HBsAg, Hepatitis B serum antigen; HCV, hepatitis C virus; NRTI, nucleoside reverse transcriptase inhibitors.
Group A: NRTI; Group B: two NRTI plus one protease inhibitor; Group C: two NRTI plus one non-NRTI.
[a]Antibodies against HCV were available for 96 individuals in group A, 194 in group B, and 14 in group C.
[b]HBsAg was available for 64 individuals in group A, 127 in group B, and 12 in group C.

was stopped. Moreover, one grade 2 and one grade 1 AST/ALT alteration cases were observed. The first was associated with a rash, with discontinuation of the drugs at day 24.

In all groups, AST/ALT levels returned to within the normal range regardless of whether they had discontinued or completed PEP.

Overall, of the 16 subjects who were anti-HCV or HBsAg positive at baseline no AST/ALT alteration was observed; all cases who developed alteration were HCV and HBV negative at baseline.

Our study suggests that AST/ALT alterations are often mild-to-moderate, and are rare during PEP regimens including two NRTI plus or minus a PI, probably because of the short duration of the prophylaxis. Indeed, studies in HIV-infected individuals taking highly active antiretroviral therapy demonstrated that hepatotoxicity usually occurs later during treatment [9–11]. Moreover, other factors that could play a role in determining highly active antiretroviral therapy-associated hepatotoxicity, such as the use of ritonavir and hepatic co-morbidity, were underrepresented in our study population.

Our study confirms previous data showing that hepatotoxicity can be more frequent and severe in nevirapine-containing regimens [12]. Because of the high incidence of severe toxicity, nevirapine use in PEP should be restricted to highly selected cases, in which resistance in the source indicates nevirapine as the sole possible alternative, with a close control of the liver function.

In all cases of AST/ALT alteration, the aminotransferase level returned within the normal range. These data suggest that, apart from nevirapine-containing regimens, the continuation of PEP appears to be safe even in case of AST/ALT alteration. Therefore, the usefulness of routine determinations of liver function tests during PEP could be re-considered.

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Directly observed antiretroviral therapy to reduce genital tract and plasma HIV-1 RNA in women with poor adherence

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Six women with substance abuse and poor adherence histories received daily antiretroviral directly observed therapy (DOT). Cervicovaginal lavage (CVL) and plasma HIV-1-RNA levels were measured at baseline, 1 month, 3 months, and 6 months. All subjects had undetectable (below 2.6 $\log_{10}$ copies/ml) CVL HIV-1-RNA levels by 3 months and undetectable plasma HIV-1-RNA levels by 6 months. The mean CD4 cell increase was 76 cells/mm^3. DOT appears effective and may reduce infectiousness in this high-risk population.

Sexual transmission is the dominant mode of HIV-1 spread throughout the world. Several factors modulate transmission risk, but the strongest biological predictor of sexual as well as mother-to-child transmission is the plasma HIV-1-RNA level [1]. As genital tract HIV-1 shedding is highly correlated with the plasma HIV-1-RNA level [2–4], increased genital tract HIV-1 shedding may be the mechanism by which high plasma HIV-1-RNA levels increase transmission. Treatment that reduces the plasma HIV-1-RNA level and genital tract HIV-1 shedding should reduce sexual and mother-to-child HIV transmission.

In clinical practice, 50% of those prescribed antiretroviral therapies are unable to realize the maximum virological benefit, primarily as a result of poor medication adherence [5–8]. Some of the many barriers to

medication adherence include side-effects, mental illness, and active substance abuse [9–16].

Building on the model of directly observed therapy (DOT) for tuberculosis, pilot programmes have begun to provide DOT for HIV-1 infection [17–21]. No previous reports have studied the impact of DOT for HIV-1 on genital tract HIV-1-RNA levels. We therefore studied the effect of DOT for HIV-1 infection on genital tract as well as plasma HIV-1-RNA levels among a small group of women with histories of poor medication adherence and recent substance abuse.

After approval by the Miriam Hospital's Institutional Review Board, individuals were recruited from the Hospital's Immunology Center. Eleven women with HIV-1 infection and self-reported substance use within the preceding 90 days were offered enrollment. All women had previously been non-adherent to antiretroviral therapy by self-report and physician judgement, and one of the proposed antiretroviral regimens was felt to be appropriate therapy. Eligible women had documented HIV-1 infection, an intact uterus, were not pregnant or wishing to become pregnant, and were free of active sexually transmitted infections. After obtaining informed consent, all subjects received stavudine 60 mg, lamivudine 300 mg, and didanosine 400 mg once a day. In addition, subjects also received either nevirapine 400 mg per day, efavirenz 600 mg per day, or a combination of saquinavir 1600 mg per day boosted with ritonavir 100 mg per day, depending on treatment histories and patient and physician preferences.

Each participant met an outreach worker daily at a location of her choosing to receive her medications, which were administered during the encounter. Each participant had an emergency supply of medications for use if meetings with the outreach worker were missed. Subjects also provided cervicovaginal lavage (CVL) and plasma specimens for quantitation of HIV-1 RNA by nucleic acid sequence-based amplification. These specimens were collected at baseline, 1 month, 3 months, and 6 months. The lower limit of detection for the nucleic acid sequence-based amplification assay was 400 copies/ml (2.6 $\log_{10}$).

Women were asked not to have vaginal sex, douche, or insert any intravaginal products for 48 h before specimen collections. Collections were deferred during menses.

CVL was collected at each study visit as described previously [22]. At baseline, each subject was screened for gonorrhoea, chlamydia, and syphilis. At baseline and at each subsequent study visit, subjects were also tested for pregnancy and for bacterial vaginosis, trichomoniasis, and candidiasis using wet mount. Each genital tract specimen was also tested for the presence of semen.

One subject died of AIDS-related complications before initiating DOT. One subject with continued virological failure had to terminate participation when HIV-1 genotyping revealed resistance to the DOT regimens. Three subjects did not follow-up for study visits. All of the remaining six subjects completed 6 months of follow-up.

Fig. 1a shows CVL HIV-1-RNA levels at each time-point of the study. At baseline, one out of six participants had HIV-1-RNA levels below the limit of detection in CVL. By 3 months, all women had undetectable HIV-1-RNA levels in CVL, with a mean decrease of 1.3 $\log_{10}$. All six subjects had plasma HIV-1-RNA levels below 400 copies/ml by 6 months, with a mean decrease of 2.4 $\log_{10}$ (Fig. 1b). Subjects also experienced a mean increase of 76 CD4 cells/mm^3.

No cases of gonorrhoea, chlamydia, or trichomoniasis were diagnosed. However, eight cases of candidal vaginitis and five cases of bacterial vaginosis were identified, with no associated increases in the CVL

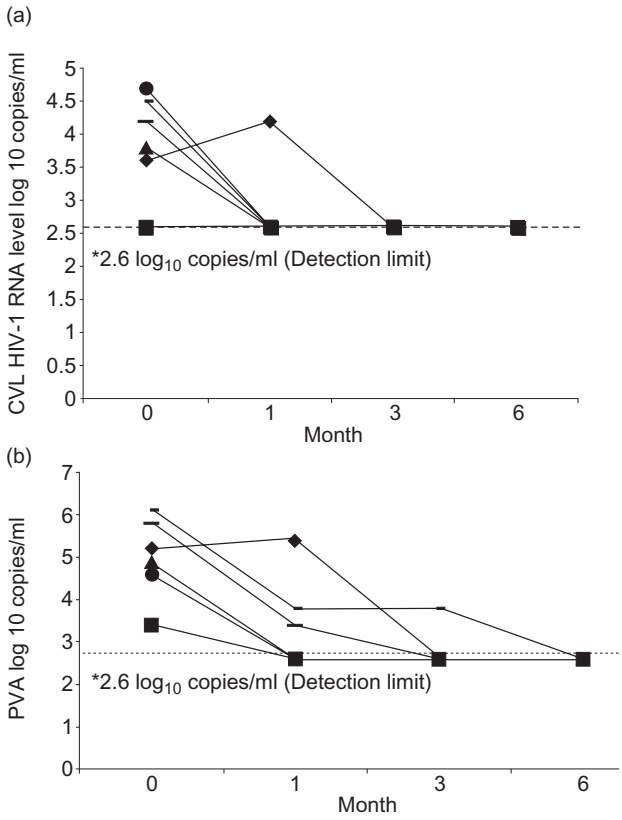

Fig. 1. HIV-RNA levels versus time. (a) Level in cervicovaginal lavage (CVL). (b) Level in plasma (PVL).

HIV-1-RNA level. One of the 24 genital tract specimens was positive for the presence of semen.

These results show that some women with a history of poor adherence and active substance abuse can realize significant benefits from antiretroviral therapy through a programme of outreach and DOT for HIV-1 infection, with decreases in both CVL and plasma HIV-1-RNA levels.

We have shown in this small group of treatment-experienced women, that multiple episodes of candidiasis and bacterial vaginosis do not seem to increase HIV-1 shedding while receiving effective therapy. This may have significant secondary prevention implications, because vaginitis is common among women with HIV-1 infection.

DOT is not universally effective or acceptable. Five of our 11 study subjects were not able to complete this programme. However, our success rate of 54% is similar to reported rates in routine clinical practice. To achieve these results among patients with previous histories of poor adherence and active substance abuse is encouraging.

Individuals with ongoing substance abuse have been shown to have high rates of unprotected sexual encounters [23–27]. DOT for HIV-1 in this population may have a significant impact on the secondary prevention of HIV-1 transmission. In addition, this programme of outreach keeps marginalized populations engaged in the healthcare system, encouraging access to other services such as tuberculosis screening, sexually transmitted diseases screening, vaccinations, and substance abuse treatment.

Its small size and short duration of follow-up limit this study. Nevertheless, it does show proof of concept, that a population of difficult to treat women can benefit from DOT when a self-administered approach fails. We have also provided some data to suggest that with successful DOT, even common genital tract infections do not lead to increases in genital tract HIV-1-RNA shedding. Larger studies are needed to confirm these findings. The development, evaluation, and refinement of observed therapy programmes are needed for all marginalized populations that are not receiving the benefits of antiretroviral therapy.

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Stavudine or indinavir-containing regimens are associated with an increased risk of diabetes mellitus in HIV-infected individuals

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Diabetes mellitus was diagnosed in 16 out of 1011 HIV-positive patients over a median follow-up of 289 days (person-year incidence 2.06, 95% confidence interval 1.18–3.33). Significant risk factors for the onset of diabetes were older age and antiretroviral therapy with stavudine or indinavir. Older men with HIV infection should be considered at higher risk of diabetes, and caution may be warranted in the use of both indinavir and stavudine in these patients.

The association between highly active antiretroviral therapy (HAART) and metabolism disorders was described shortly after the introduction of HIV-1 protease inhibitors (PI) into routine clinical practice [1]. Since then, the complex syndrome of hyperlipidemia, lipodystrophy or diabetes mellitus (DM) has been observed in a considerable proportion of patients [2], leading to an increased risk of morbidity and mortality as a result of metabolic disorders and coronary heart disease [3], increased diagnostic and therapeutic costs, and significant patient discomfort secondary to body image alterations.

It has been reported that hyperglycemia with or without DM occurs in 3–17% of patients receiving HAART, shortly after the start of PI or even after their prolonged use [4]. Whether this effect is a direct result of PI therapy or a PI-exacerbated primary HIV metabolic defect is still unclear [5]. At present, most data concerning the incidence of diabetes and its correlation with antiretroviral therapy (ART) come from cross-sectional studies of small numbers of patients.

In the present study, we have analysed retrospective data from a cohort of 1011 HIV-positive patients (68% men, median age 37 years, range 18–74, median duration of known HIV infection 84 months, range −3 to 210 months); clinical, biochemical, immunological, virological and therapeutic data were collected from an in-house database, started on 1 November 1999. Blood tests were obtained for each patient at regular intervals as a part of routine outpatient care.

Study patients met the following criteria: normal fasting plasma glucose levels at study entry, and no previous diagnosis of DM; at least two fasting plasma glucose determinations during follow-up; a follow-up of at least 3 months; a stable (without interruption) antiretroviral regimen (or no therapy) for at least one month before entry and throughout the follow-up period.

Follow-up was censored at the last available plasma glucose assay or at the diagnosis of DM; the whole exposure time to each drug since its start was also recorded. DM was diagnosed on the basis of the 1997 American Diabetes Association guidelines (fasting plasma glucose $\geqslant$ 126 mg/dl on two different occasions) [6].

Data are reported as median values (minimum, maximum). As the variables did not have a Gaussian distribution, the Mann–Whitney test was used to investigate between-group differences. Cox proportional hazard regression models were used to investigate the associations between DM and putative risk factors (age, sex, CD4 cell count, HIV-RNA level, antiretroviral drugs).

During the follow-up (median 289 days, 91–624 days) DM was diagnosed in 16 out of 1011 patients [person-year incidence 2.06, 95% confidence interval (CI) 1.18–3.33]. Table 1 summarizes the statistical analyses

Table 1. Risk factors for diabetes onset in 1011 HIV-positive patients: features of diabetic patients.

	Diabetic patients (n = 16)	Other (n = 995)	*P* values
Male sex (%)	87.0	67.9	0.09
Age (years)	47 (36–72)	37.7 (18–74)	0.0001
CD4 cell count (cells/μl)	498 (217–1339)	530 (6–2318)	0.8
Viral load (copies/ml)	470 (19–16 000)	410 (19–960 000)	0.6
On therapy (%)	94	81	0.2
Whole exposure to			
Antiretroviral drugs (days)	371 (30–1315)	603 (30–3655)	0.23
PI therapy (%)	80	66	0.25
Fasting cholesterol (mg/dl)	196 (138–282)	nd	–
Fasting triglycerides (mg/dl)	339 (121–803)	nd	–

PI, Protease inhibitor.

of putative risk factors for DM. Older age [hazard ratio (HR) 1.11, 95% CI 1.06–1.16, $P < 0.001$] was associated with a higher risk of developing diabetes, whereas male sex showed a trend towards it, without reaching conventional statistical significance (HR 6.4, 95% CI 0.83–48.8, $P = 0.07$).

After multivariate analysis adjusted for age and sex, diabetes onset was unrelated to the CD4 cell count, HIV-RNA level, ART assumption as a whole, PI therapy versus non-PI-containing regimens. Of note is the fact that there was no statistically significant difference in the whole duration of ART between diabetic and non-diabetic patients, thus including the pre-enrollment time. Furthermore, age and sex-adjusted Cox regression models of the individual drugs showed that patients treated with stavudine or indinavir were at significantly higher risk of developing DM (stavudine: HR 16.0, 95% CI 3.03–83.8, $P = 0.001$; indinavir HR 4.0; 95% CI 1.26–12.7, $P = 0.018$) (Table 2). To address the issue of whether the onset of diabetes may be stochastic or dose dependent, we investigated whether exposure to stavudine or indinavir (in the whole cohort) was significantly longer compared with other drugs. We found a potential confounding bias in the case of abacavir, efavirenz or nelfinavir because these drugs were registered later in Italy. However, the median duration of exposure was similar among stavudine and other reverse transcriptase inhibitors, as it was among indinavir, saquinavir and ritonavir (data not shown). We therefore tried to understand a possible synergic role of stavudine and indinavir therapy in the onset of DM, since after the multivariate analysis in the subgroup of patients taking indinavir alone did not show a significant HR for the development of diabetes (HR 16, 95% CI 0.26–75.4, $P = 0.3$), whereas patients on stavudine alone (HR 16.5, 95% CI 2.23–121.6, $P = 0.006$) and those on stavudine and indinavir were at significantly higher risk (HR 16.5, 95% CI 5.0–314.9, $P < 0.0001$). Finally, fasting cholesterol and triglyceride levels in index cases were collected close to the time of diabetes diagnosis (Table 1).

Table 2. Risk factors for diabetes onset in 1011 HIV-positive patients: hazard ratio for diabetes onset, Cox regression analysis of single antiretroviral agents.

Drug	Hazard ratio[a]	95% CI	*P*
Zidovudine	0.22	0.05–1.02	0.052
Didanosine	1.4	0.17–11.14	0.75
Lamivudine	1.8	0.51–6.37	0.36
Stavudine	16.0	3.03–83.8	0.001
Nevirapine	1.92	0.41–8.96	0.41
Saquinavir	1.58	0.34–7.34	0.56
Indinavir	4.0	1.26–12.7	0.018
Ritonavir	1.02	0.13–8.0	0.98
Nelfinavir	0.93	0.28–3.08	0.91

[a]Hazard ratios for zalcitabine, abacavir and efavirenz were similar to other drugs (except stavudine and indinavir); the confidence intervals (CI) for these drugs were not calculated because of the very small number of patients on therapy.

The present study has two limitations, the first being the small group of index cases and the second that cohort patients were already on antiretroviral drugs at study enrolment; in this regard our data should be confirmed in large cohorts of antiretroviral-naive patients [7]. Despite that, this is to our knowledge the first longitudinal study about diabetes incidence in such a large cohort of HIV-positive patients. Very few studies investigating the incidence of diabetes in the general population have been performed, even in Italy. One study in a population in northern Italy [8] estimated a yearly incidence of 2.2 per 1000 person-years. The population was older than the HIV cohort we have followed, therefore we cannot draw any conclusion from this, but we may speculate whether some antiretroviral drugs can trigger the onset of diabetes in predisposed individuals. Despite that, the results about the drug-related risk of diabetes were partly surprising. First, we clearly showed an increased risk of DM in patients on indinavir, thus confirming preliminary results from in-vitro and in-vivo studies, occasionally leading to some pathogenic models [9,10].

As for stavudine, this is the first report suggesting that this drug may be an independent and strong risk factor for the onset of DM. At present, data are both anecdotal and controversial with regard to the causative role of single nucleoside reverse transcriptase inhibitors (didanosine and abacavir) in diabetes onset [11,12], whereas data on lipoatrophy/lipodystrophy and hypertriglyceridemia after stavudine use are as yet inconclusive [10–12]. As a result of our epidemiological results we cannot speculate about a pathogenic model for stavudine-related DM, but we are concerned about the risk of diabetes in patients on prolonged stavudine or indinavir therapy. In this regard, we propose that older men with HIV infection are considered at higher risk of DM, and that caution may be warranted in the use of both indinavir and stavudine in these patients.

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Highly active antiretroviral therapy in resource-poor settings: the experience of Médecins Sans Frontières

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We describe the short-term results of highly active antiretroviral therapy (HAART) in seven projects in low and middle income countries. A total of 743 adults were included, and clinical, immunological and virological responses were analysed. At 6 months, outcomes were similar to those observed in western countries, and the probability of remaining on treatment was 94%. The challenge now is to extend access to HAART to the millions in urgent need.

Médecins Sans Frontières has initiated highly active antiretroviral therapy (HAART) programmes in various settings in resource-poor countries over the past 2 years. After adherence consultations to explain the treatment and its constraints, HAART was proposed to severely immunocompromised patients following the World Health Organization (WHO) recommendations [1]. Only triple-therapy regimens were proposed, mainly including a non-nucleoside reverse-transcriptase inhibitor (NNRTI) as preference was given to the easiest scheme to follow, with the lowest pill burden. The CD4 cell count was measured on admission and every 6 months. The HIV viral load was systematically measured when possible (three projects). Antiretroviral supply relied on local market competition, including the use of quality-assured generics. Treatment was provided free to patients.

As of May 2002, 881 patients had started HAART in seven projects (Chiradzulu District Hospital, Malawi; Homa Bay District Hospital, Kenya; Khayelitsha Health Centers, South Africa; Institut de Recherche et Développement, Military Hospital, Yaoundé, Cameroon; PBN Sihanouk Hospital, Phnom Penh, Cambodia; Surin Provincial Hospital, Thailand; and Roosevelt Hospital, Guatemala City, Guatemala). This first analysis aimed at describing the inclusions and short-term outcomes after compiling data from all the projects.

All patients aged 14 years or more, followed under HAART, were included in the analysis. Individual data systematically collected were sex, age, treatment back-

ground, dates of starting HAART, last visit or death, WHO clinical stage and regimen prescribed at baseline, CD4 cell count and HIV viral load at baseline and at 6 months if available. Kaplan–Meier methods were used to estimate the probability of survival and of remaining on treatment. At 6 months, any change in weight and CD4 cell counts were analysed, as well as the proportion with undetectable viral loads. Pooled results are presented and the extreme ranges among projects.

A total of 743 adults were included (median age 33 years, 50.3% women); 61 children and 77 adults just beginning HAART were excluded. Patients were at an advanced stage at the start of therapy (Table 1). The median CD4 cell count on inclusion (n = 684) was 48 cells/mm^3 (25–75th percentiles: 11–120). The median baseline viral load, available for 231 patients, was 132 000 HIV copies/ml (5.12 $\log_{10}$ copies/ml; 25–75th percentiles: 4.56–5.52). Among the 432 patients for whom the information was available, 339 (78.5%) were antiretroviral naive. Overall, 620 patients (83.4%) initiated NNRTI-containing regimens and 123 (16.6%) protease inhibitor-containing regimens. The median period of observation was 4.0 months (25–75th percentiles: 1.7–6.9). During this period, 61 patients died (of whom 26 died within the first 30 days; 42.6%), 25 stopped treatment (for adherence problems or patient request), and 18 were lost to follow-up for 60 days or more (Table 1). Overall, 240 patients (32.3%) were on treatment for 6 months or more. The probability of survival at 6 months was estimated at 89.5% [95% confidence interval (CI) 86.8–92.1]. Among those surviving, the probability of remaining on treatment at 6 months was estimated at 94.0% (95% CI 91.8–96.1). Among 200 patients controlled at 6 months, the median increase in the CD4 cell count was 104 cells/mm^3 (25–75th percentiles: 47–163) (Table 1). Among 118 patients controlled for viral load at 6 months, 106 (89.8%) were undetectable (below 500 copies/ml). A total of 110 patients (14.8%) changed regimen. The reasons were systematically assessed in four projects (56 patients switching): 39 (69.6%) for intolerance [25 zidovudine (24 anaemia, one myopathy), six indinavir/ritonavir (two urinary lithiasis, two gastrointestinal disorders, four hyperlipidemia), five nevirapine (three rash, two hepatitis), two efavirenz (dizziness), one didanosine and one stavudine (both for neuropathy)]; 17 (30.4%) for other reasons (tuberculosis treatment in 15 cases).

These preliminary results show the major benefits brought by HAART in resource-poor settings, even for patients at an advanced stage of AIDS. Patients show regular attendance, and immunological and virological responses indirectly indicate a high level of individual drug adherence. This first pooled description also highlights differences between projects in baseline characteristics and, consequently, outcomes (Table 1). A comparative analysis of prognosis factors is limited by the short follow-up period. Opportunistic infections occurring under HAART were not described. The main problems encountered in our experience are the occurrence of tuberculosis in Africa and Asia, of severe fungal infections in Guatemala and the persistence of Kaposi's sarcoma in Africa.

Currently, 6 million people in developing countries are in need of treatment. Regardless of the need for operational research, our results support the belief that treating severely immunocompromised AIDS patients is feasible in various settings, including in peripheral health facilities, as has also recently been reported by Ugandan and Senegalese national initiatives [2,3]. Technical issues need to be addressed urgently in order to scale up access to HAART. Greater access to HAART will be facilitated by the ongoing price reduction of antiretroviral drugs. Triple therapy is currently available at US$361 per year from quality

Table 1. Characteristics of 743 adults at the start of highly active antiretroviral therapy and response to therapy.

		Range among projects	
	All projects	Minimum	Maximum
Baseline characteristics			
Number of adults starting HAART (n)	743	62	154
Proportion at WHO clinical stage 4 (%)	56.3	28.4	78.6
Median CD4 cell count (cells/mm^3)	48	10	151
Proportion prescribed NNRTI	83.4	34.2	100
Outcomes during the period of observation			
Proportion of deaths (%)	8.2	0.0	18.8
Proportion of deaths after 30 days therapy (%)	4.7	0.0	8.6
Proportion lost to follow-up (%)	2.4	0.0	5.2
Restoration after 6 months on treatment			
Median weight gain (kg) (n = 228)	3	1	6
Median CD4 cell count gain (cells/mm^3) (n = 200)	104	79	139

HAART, Highly active antiretroviral therapy; NNRTI, non-nucleoside reverse transcriptase inhibitor; WHO, World Health Organization.

generic suppliers [4]. This price should come down to as low as US$50 per year. New fixed-drug combinations (in one pill) are also needed to improve patients' adherence and the management of antiretroviral therapy in peripheral health structures. Besides treatment protocols, monitoring should be simplified and adapted to each context [5,6]. Pilot projects using clinical and biological proxy markers instead of CD4 cell counts and viral loads should be developed as a priority, together with the reinforcement of health structures to ensure access to testing and the availability of trained staff.

Funding HAART programmes requires a huge international effort to meet the growing political commitment in affected countries. As of July 2002, the Global Fund to Fight AIDS, Tuberculosis and Malaria effectively received only US$616 million for 2 years compared with the estimated US$10 billion per year needed to fight AIDS alone. Above all, increasing access to treatment is a challenge requiring strong political and financial commitments from both developed and developing countries.

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Definition of loss of virological response in trials of antiretroviral drugs

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Definition of the time of loss of virological response is important when designing a viral load endpoint for trials of antiretroviral drugs. We assessed whether, in patients who achieved a viral load below 50 copies/ml, two consecutive values above 50 copies/ml was really indicative of loss of response. It was common for the viral load to return to below 50 copies/ml with no change in regimen, suggesting that a higher threshold for defining the loss of response is required.

The recently published Food and Drug Administration (FDA) guidelines for the analysis of trials of antiretroviral drugs suggest that those who have experienced a virological response (two viral load values below the lower assay quantitation limit; i.e. 50 copies/ml) should be defined as having a 'loss of virological response' if two consecutive values above 50 copies/ml are recorded [1]. To now, the more commonly used thresholds to define the loss of virological response are consecutive values above 200 or 500 copies/ml, because of the concern that transient increases in the viral load value above 50 copies/ml, whether caused by assay variability or real fluctuations, are common [2–5]. To evaluate the FDA criterion, we assessed the subsequent viral load values in 376 individuals from the Universitat Clinic in Frankfurt who had experienced consecutive values below 50 copies/ml but then later experienced two values above 50 copies/ml. Those with any change in therapy were excluded. At the time of achieving a viral load below 50 copies/ml all patients were on at least three antiretroviral drugs, including at least two nucleoside analogues. The median (interquartile range; IQR) viral loads for the first and second values above 50 copies/ml were 565 copies/ml (140–9000) and 600 copies/ml (170–7190), respectively (median 35 days between these values). The next viral load value was taken a median of 35 days later. In 86 individuals (23%) this viral load was below 50 copies/ml, despite there being no change in the antiretroviral

regimen [median (IQR) viral load 500 copies/ml (70–6950)]. When we further restricted analysis to those 135 individuals for whom the two loss-of-response-defining values above 50 copies/ml were both between 51 and 500 copies/ml, 56 (42%) had the next value below 50 copies/ml. For comparison, we considered other viral load thresholds for defining loss of response. Using 200 copies/ml, 36 out of 296 individuals (12%) who fulfilled the definition of loss of response had the next viral load value below 50 copies/ml, whereas for 500 copies/ml, this was nine out of 228 individuals (4%). These results are consistent with previous findings [4,5], and suggest that many patients who fulfil the FDA definition of loss of virological response may not, in fact, have lost that response and that a higher threshold may be more appropriate.

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Natural killer cells are not infected by Kaposi's sarcoma-associated herpesvirus *in vivo*, and natural killer cell counts do not correlate with the risk of developing Kaposi's sarcoma

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Although the innate immune system is implicated in the control of Kaposi's sarcoma (KS), the risk of developing KS is not associated with the nadir natural killer (NK) cell count, and NK cell counts do not significantly increase or decrease during KS resolution. KS-associated herpesvirus replication was not demonstrated *in vivo* or *in vitro* within NK cells, suggesting that NK cells do not contribute to the resolution of KS. Their role appears limited to events occurring during early infection.

Natural killer (NK) cells mediate non-adaptive responses against virus-infected cells and modulate the activity of other effector cells of the adaptive and innate systems [1]. In particular, NK cells have been shown to kill those target cells that have lost or express low levels of MHC class I molecules, a frequent event in tumour or virus-infected cells [2]. In common with many viruses, Kaposi's sarcoma-associated herpesvirus (KSHV) achieves evasion of host T-cell recognition by the downregulation of MHC class I in infected cells [3,4]. In theory, this leaves infected cells susceptible to NK cell lysis, but the discovery of a class I homologue, *UL18*, in the genome of cytomegalovirus, a related herpesvirus, has led to speculation that such viral proteins may serve as NK cell decoys [5].

Recently, cells latently infected by KSHV have been shown to be efficiently lysed by NK cells from individuals with a normal immune response, and this NK cell activity was found to be significantly reduced in AIDS patients with progressing KS compared with normal blood donors and HIV-positive individuals with KS that responded to highly active antiretroviral therapy (HAART) [6]. As persistent HIV-1 infection of NK cells in patients receiving HAART has been shown and is postulated to represent a latent HIV-1 reservoir [7], it is conceivable that the increase in NK cell activity after successful antiretroviral treatment in KS patients may be related to an effect on HIV-1. We therefore investigated the relationship between the development of KS and the NK cell count, and established whether NK cells are infected by KSHV *in vivo*.

NK cell counts, defined as the CD16/CD56 count, were linked to 5810 individuals from the Chelsea and Westminster HIV-1 cohort. The nadir NK cell count was defined as the lowest ever NK cell count recorded during patient follow-up to the time of KS diagnosis [NK cell count < 17 cells/mm^3, rate ratio 1.01, 95% confidence interval (CI) 0.67–1.53, for a NK cell count > 71 cells/mm^3, rate ratio 1.0]. Person-days of follow-up were converted to person-years at risk (PYAR). In order to keep the coefficient of PYAR constant, this was log transformed and used as the offset in the Poisson regression. The data were analysed using the Genmod procedure in SAS version 8.0 with log

link and Poisson error distributions. PYAR was estimated from entry into the cohort to either end of the study period, the development of KS, the last recorded visit, or if the patient had died during their follow-up then their date of death. One hundred patients with KS that resolved during the HAART era were also identified, and their NK cell counts were recorded from the time of KS diagnosis every 3 months for one year.

To establish the presence of KSHV in NK cells, between January and March 2003, peripheral blood mononuclear cells (PBMC) were provided by eight consecutive patients with KS. PBMC were separated by a Ficoll-Histopaque gradient, NK cells were isolated by negative selection and NK-depleted PBMC by positive selection, using an antibody-based purification technique (Miltenyi Biotech, Bergissch Gladbach, Germany). The purity of the cells after column depletion ($> 95\%$) was determined by flow cytometry.

In order to identify KSHV, DNA was extracted from 2×10^6 NK cells (or NK-depleted PBMC) and polymerase chain reaction (PCR) for KSHV gene fragments was performed, as previously described [8]. To determine whether KSHV could infect NK cells *in vitro*, we incubated 2×10^6 uninfected NK cells ($KSHV^-HIV^-$ by PCR) with 3×10^7 KSHV particles, with and without increasing doses of IL-2 (20 U/ml, 50 U/ml and 100 U/ml). KSHV was prepared as by lysis and ultracentrifugation of lysed BC-3 cells, a KSHV-positive cell line derived from a primary effusion lymphoma and competitive PCR was used to determine KSHV titre [9]. After 48 h, NK cells were washed and recultured for a further 4 days before DNA extraction.

There was no association with the risk of developing KS and different levels of nadir NK cell counts (Table 1; likelihood ratio χ^2 test, $P = 0.6$). In individuals with KS resolution during HAART, there were no statistically significant changes in the NK cell count or percentage (Fig. 1), and there were also no notable changes in the NK cell count when these patients were stratified according to HIV-1 virological failure, CD4 cell count or type of therapy (data not shown).

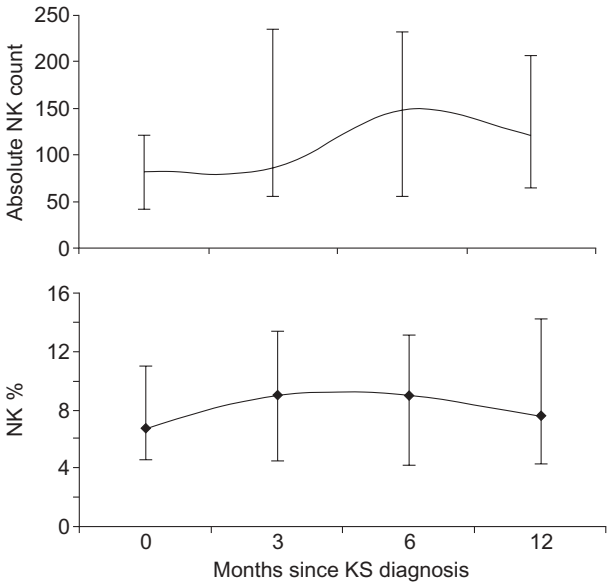

Fig. 1. Changes in natural killer cell count and natural killer cell percentage in 100 individuals with Kaposi's sarcoma resolution during the era of highly active antiretroviral therapy. The median and interquartile range is shown. KS, Kaposi's sarcoma; NK, natural killer.

In eight consecutive patients with histologically confirmed KS, NK cells were derived from PBMC. These individuals had a wide range in their ages, CD4/CD8 cell counts, HIV-1 viral loads and treatment. PCR to detect KSHV was negative in all eight NK cell populations tested (Fig. 2, lanes 8–16) and positive in the NK-depleted PBMC from the same patients (Fig. 2, lanes 1–8). Although we have demonstrated the infection of NK cells from normal donors by high titres of KSHV *in vitro* (with or without IL-2), we were unable to establish the presence of any KSHV replica-

Table 1. Risk of developing Kaposi's sarcoma according to natural killer cell count (quartiles up to the lower end of normal shown).

Nadir NK count (cells/mm^3)	Incidence of KS per 1000 patient-years	Rate ratio and 95% CI
< 17	1.8	1.01 (0.67–1.53)
17–35	2.0	1.10 (0.73–1.66)
36–70	1.9	1.03 (0.68–1.59)
> 71	1.8	1

The number of Kaposi's sarcoma (KS) cases, stratified according to the natural killer (NK) cell count is shown with the percentage of the total patient population in brackets.
The incidence of KS per 1000 patient-years and the rate ratio with 95% confidence intervals (CI) are shown ($P = 0.603$ using likelihood ratio χ^2 test).

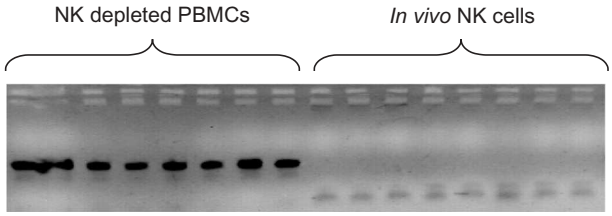

Fig. 2. Kaposi's sarcoma-associated herpesvirus polymerase chain reaction products on a 1.5% agarose gel. Natural killer (NK)-depleted peripheral blood mononuclear cells (PBMC) from patients 1–8 are in the left-hand eight lanes and are all positive. Kaposi's sarcoma-associated herpesvirus gene products were not amplifiable from DNA extracted from patients' NK cells (lanes 9–16).

tion in NK cells, as shown by subsequent limiting dilution PCR (not shown).

Previous data have suggested that the restoration of immune responses during HAART may be particularly effective in the therapy of early KS as a result of the control of latent KSHV infection by NK cells [6]. In our group of eight patients, we were unable to show KSHV infection of NK cells *in vivo*, and we suggest that the effect of HAART on HIV-1 infection of NK cells, resulting in improved cytolytic activity, appears to be a more likely explanation for the observed results. This may indicate that NK cells lack the cellular environment that facilitates gammaherpesvirus replication. Whereas recent research has concentrated on the development of an effective T-lymphocyte response as a result of HAART, this may not be the mechanism for tumour regression in individuals with KS. Despite the data shown, we believe that a more extensive study may elucidate the role of NK cells or other aspects of the innate immune system in the control of KS, in particular in initial events in infection and tumorigenesis.

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