

Correspondence

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Is post-exposure prophylaxis affordable?

Although a previous analysis has shown that the use of antiretroviral drugs for post-exposure prophylaxis (PEP) against HIV after suspected sexual exposure could be cost-effective, if restricted to regular partners of HIV-infected persons, to patients reporting unprotected receptive anal intercourse (including condom breakage), and possibly to cases in which there is a substantial likelihood that the partner is infected [1], the careful analysis by Low-Beer and colleagues [2] of the cost of providing PEP to high-risk men who have sex with men (MSM) in Vancouver British Columbia's West End is the first study to examine the affordability of this strategy. Their analysis indicates that making PEP available to this relatively small population of men could quickly exhaust available funding.

We have done some simple calculations to determine the affordability of providing PEP in the USA at the national level, and arrived at a similar conclusion. The 1997 budget for the US Centers for Disease Control and Prevention's (CDC) national HIV prevention program was just over US\$600 million [3]. If this money were instead directed solely at PEP programs, it would pay for approximately 550 000 treatments (at a cost of US\$1092 for dual-drug PEP), which would prevent approximately 880 HIV infections in a high-risk MSM population with a 20% prevalence of HIV, assuming that PEP is 80% effective and that the probability of HIV transmission is 1% (more infections could be prevented if PEP were restricted to partners of men known to be infected). This represents only a small proportion of the 40 000 new HIV infections that occur in the USA each year [4]. Although the number of infections that would occur in the absence of the

CDC national prevention efforts is unknown, it is believed to exceed the relatively small number that could be averted through PEP alone [3].

Depending on the demand, making PEP available to persons who have experienced a potential exposure to HIV through sex or drug injection could be extremely expensive. The simple analysis presented above suggests that this money might be better spent on other strategies to prevent the spread of HIV [2,5].

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A reality check: the cost of making post-exposure prophylaxis available to gay and bisexual men at high sexual risk

The successful use of antiretroviral therapy for post-exposure prophylaxis (PEP) in cases of accidental and occupational exposures has prompted researchers, clinicians and public health decision makers to ask whether PEP would be effective in cases of sexual exposures. Although the answer to this question remains unknown, some factors that need to be considered have been identified. These include: the frequency of exposure, the probability of knowing the HIV status of the source, the elapsed time between exposure and

possible intervention, and the impact PEP may have on risk reduction behaviours. In addition, given the limited resources available for the management of HIV disease, the cost of PEP will play a certain role in deciding its future.

Other studies [1–3] have investigated whether using PEP for sexual exposures to HIV is effective. Pinkerton *et al.* [3] conducted an in-depth cost–utility analysis, and concluded that PEP should be recom-

mended to partners of infected persons, to patients reporting unprotected anal intercourse, and possibly in cases in which there is a substantial likelihood that the partner is infected. In terms of clinical practice, no consensus has been established among physicians on the use of PEP for non-occupational exposures. Although some physicians are reluctant to prescribe antiretroviral prophylaxis, others appear to have a favourable attitude towards the prescription of PEP for sexual exposures [4]. For example, in San Francisco, CA, USA, two clinics have recently opened and are currently providing anti-HIV prophylaxis after high-risk sexual exposures [5,6]. In the light of the uncertainty surrounding this issue and its possible implications, leading commentators in the area have made a call for the development of rational guidelines [4,7].

The following cost analysis is a step in this direction because the wise allocation of scarce resources is an integral part of any HIV management strategy. Although a few economic analyses have been conducted, no studies have investigated how much it would cost to provide PEP to a known at-high-sexual-risk population.

The purpose of our study was to determine the cost of providing post-exposure prophylaxis to the male gay and bisexual community at high sexual risk of contracting HIV in the West End of Vancouver, British Columbia, Canada. A cost estimate was obtained by multiplying the cost of antiretroviral prophylaxis per course by the number of gay and bisexual men at high sexual risk and by the number of risk incidents per person per year. To determine how many men were at high risk, responses on a self-administered questionnaire given to a prospective cohort of gay and bisexual men beginning in May 1995 were used. High sexual risk was defined as having at least one episode of unprotected anal sex (insertive or receptive) with a casual male partner in the previous year, or having at least one episode of unprotected anal sex (insertive or receptive) with an HIV-positive man in the previous year. The proportion of men in the West End who identified themselves as either gay or bisexual was derived from a random telephone survey, and the cost of a course of PEP was taken from the British Columbia Centre for Excellence in HIV/AIDS Drug Treatment Program. Monte Carlo methods were used to simulate confidence limits around the cost estimate.

Out of an estimated total of 5100 (95% confidence interval: 4700–5400) gay and bisexual men in Vancouver's West End, 1391 (27.3%) were classified as being at high risk of contracting HIV through unsafe sexual behaviours on the basis of prospective cohort data. The average number of risk incidents per

person per year was three (0–6) and, depending on the recommended regimen, stavudine and lamivudine or triple therapy with nelfinavir, the average cost of PEP varied from Can\$530 to 903 with an average price of Can\$560. On the basis of these assumptions and the Monte Carlo simulation, the potential cost of making post-exposure prophylaxis available to all those at high sexual risk in the West End was estimated to be Can\$2 259 780 (95% confidence interval: Can\$800 000–4 100 000).

The above minimum cost estimate, Can\$800 000 per annum, for providing PEP to at-risk gay and bisexual men in Vancouver's West End is approximately equal to British Columbia's current budget for all accidental exposures in the province. This is an important fact considering the limited resources available to fight HIV disease and Canada's universal healthcare system. In light of the numerous uncertainties regarding the effectiveness of PEP for sexual exposures, the growing cost of providing anti-HIV therapy for confirmed positive individuals, and the potential cost of expanding the use of PEP to include high-risk consensual sex, we feel that other preventative strategies should take priority. Future research in this area is needed so that clinicians, researchers, policy makers and HIV-positive persons can gain a better sense of the issues surrounding and the implications of making PEP available for sexual exposures.

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Diversity of HIV-1 genetic subtypes in France, in the context of mother-to-child transmission

HIV-1 displays a high genetic variability that is rapidly evolving. Phylogenetic analyses have resulted in the classification of HIV-1 isolates in two groups, group M (major) and group O (outlier) [1]. Group M viruses (subdivided into 10 subtypes: A–J) are widespread; in contrast group O viruses are mainly found in patients from central Africa or in patients originating from that region. In western Europe, the AIDS epidemic is mainly caused by the B subtype, although a co-circulation of non-B subtypes has recently been reported in Belgium, Sweden, the United Kingdom and the Netherlands [2–5]. In France, non-B subtypes have been isolated in individuals originating from Africa and in caucasian patients [6]. Moreover, the prevalence of non-B strains in French blood donors increased between 1985 (4%) and 1995 (20%) [7]. The aim of this study was to describe HIV-1 subtype repartition in the context of mother-to-child transmission, within a group of individuals living in France and originating from Africa. In the French National Cohort Study, a progressive evolution of the percentage of African mothers infected with HIV-1 has been observed; it was approximately 10% in 1990 and 40% in 1997 [8]. Three mothers infected with HIV-1 group O viruses have also been identified and one case of transmission to the child has been described [9]. In the present study, all available samples collected in the period 1986–1996 from patients originating from Africa were selected, and thus 163 HIV-1 strains were analysed from 70 infected infants and from a further 93 mothers who gave birth to non-infected children. Genotyping was performed by the heteroduplex mobility assay as described by Delwart *et al.* [10]. DNA was extracted from peripheral blood mononuclear cells, and the polymerase chain reaction was performed using ED3–ED12 as outer primers and ES7–ES8 as inner primers.

The repartition of HIV-1 variants, identified in the 18 corresponding countries, is presented on the map in Fig. 1. For seven African patients, the country of origin was unknown; the repartition of subtypes was six A and one D. In west Africa, subtype A viruses were predominant (41/54, 76%). A more complex subtype distribution was observed in central Africa. Among the 101 infected patients, all the main HIV-1 subtypes were identified (56 A, 13 B, 4 C, 10 D, 2 E, 6 F, 7 G, 3 H). This study, performed on a large number of samples, completes the results described by several studies conducted locally in each African country [11–13]. Subtype A was found in all parts of west and central Africa and the largest diversity of HIV-1 subtypes was found in central Africa, indicating a large co-circulation of variants in these countries. However, we reported 13% of HIV-1 B subtype, which seems higher than previously reported either in west or central Africa. It was difficult to determine whether these mothers had been infected before or after moving

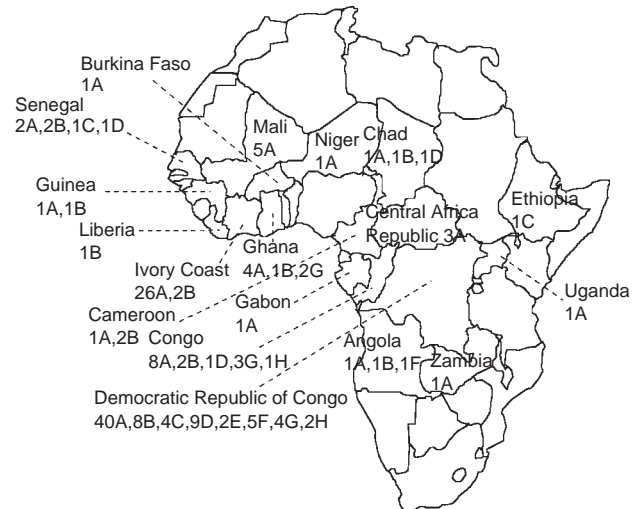


Fig. 1. Map of Africa: the repartition of the viral subtypes is indicated for each of the 18 countries.

to France. However, they are likely to have been infected with African strains because they have been living in France for less than 2–3 years and they mostly stay within their own community.

Therefore, as early as 1986, a very high diversity of HIV-1 subtypes was circulating among the community of African mothers living in France. This emphasizes the increasing importance of viral strains trafficking in France. It has been reported that HIV-1 subtype diversity may have a major impact on viral load quantification [14,15]. Therefore, it is important to be aware of this viral diversity existing in France, to avoid viral load misquantification in pregnant women and the misdiagnosis of HIV-1 infection in babies. In our laboratory, diagnosis is performed using HIV-1 polymerase chain reaction DNA on peripheral blood mononuclear cells with two different pairs of primers, selected within conserved genes (*LTR* and *gag*) in order to be able to detect the majority of viral strains.

At present, in the French National Cohort Study, no difference in HIV-1 transmission rates has been found between Caucasian and African mothers [8]. In conclusion, the periodic evaluation of circulating viral strains is necessary for epidemiological surveys; moreover, such studies should allow the characterization of sufficient samples to address the question of the eventual differences in the pathogenicity of the different viral subtypes, particularly in the context of mother-to-child transmission.

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HIV virions and HIV replication are unaffected by granulysin

Granulysin is a small peptide commonly found in the granules of CD8 T cells and natural killer (NK) cells [1,2]. A role for granulysin has been implicated in the elimination of certain intracellular microorganisms by CD8 T cells [2]. It is a member of the saposin-like protein family of lipid-binding proteins [1,3]. Saposins interact with lipid membranes and activate lipid-degrading enzymes such as glucosylceramidase and sphingomyelinases, which can lead to apoptosis by initiating the caspase cascade [4–6]. Recombinant granulysin shows a dose-dependent growth inhibition of several bacteria including *Mycobacterium tuberculosis*, *Salmonella typhimurium*, *Escherichia coli*, *Listeria monocytogenes*, and *Staphylococcus aureus* [7]. In addition, it can kill the fungi and parasite, *Cryptococcus neoformans*, *Candida albicans*, and *Leishmania major*. To date, however, nothing is known about the antiviral properties of granulysin.

HIV is an enveloped virus that, like all viruses, replicates intracellularly. It is susceptible to elimination by cytotoxic T lymphocyte (CTL) killing of infected cells [8]. However, little is known about whether the HIV-infected cells release infectious virions upon lysis by CTL. An agent that could cause the direct inactivation

of the HIV virions would further control the spread of infection. Because granulysin is released by CD8 T cells upon antigen recognition, and has the above-mentioned antimicrobial properties, particularly its lipid membrane disrupting nature, we examined the potential antiviral activity of recombinant granulysin against HIV.

To assess the possibility of a direct antiviral effect by granulysin on HIV particles, recombinant granulysin [9] was added to fluids containing 600 and 3000 TCID₅₀ of HIV-1_{SF2} [10] diluted in serum-free AIM-V medium (Gibco-BRL, Gaithersburg, MD, USA), to yield 5 mM of granulysin. The mixtures were incubated for 2–3 h at 37°C before being used to inoculate phytohemagglutinin-P (3 µg/ml) (Sigma Chemicals, St Louis, MO, USA)-stimulated CD4 cells from an HIV-seronegative control subject. After 1 h, the cells were washed and 3 × 10⁵ infected cells were cultured in a 48-well culture plate (in triplicate) in complete medium, which consisted of RPMI 1640 medium containing 10% heat-inactivated (56°C for 30 min) fetal calf serum, 1% antibiotics (100 U/ml penicillin; 100 µg/ml streptomycin) 2 mM glutamine, and 100 U/ml recombinant IL-2 (Glaxo Wellcome,

Research Triangle Park, NC). The cultures were passed and monitored for reverse transcriptase (RT) activity [11] every 3 days.

Treatment of HIV virions with recombinant granulysin did not appreciably alter their infectivity, as indicated by similar peak levels of virus replication (Table 1) and replication kinetics (data not shown), relative to the control untreated virion cultures.

We next evaluated whether culturing acutely infected CD4 T cells in the continued presence of granulysin would affect HIV replication. Phytohemagglutinin-P-stimulated CD4 T cells were inoculated with approximately 3000 TCID₅₀ of HIV-1_{SF2}. After incubation for 1 h, the cells were washed and plated (in triplicate) in 96-well culture plates (10⁵ cells/well) in complete medium. The infected CD4 cells were cultured alone or in the presence of various concentrations of granulysin (diluted in RPMI 1640 medium). Granulysin was added at the initiation of culture and replenished every 2 days thereafter upon cell passage. The amount of HIV replication in the cultures was determined every 2 days by measuring RT activity in culture fluid samples.

In four separate experiments, granulysin showed no substantial effect on the levels of HIV production relative to the untreated control, and in general, no appreciable effect on cellular viability. In the different experiments, the peak levels of HIV replication reached between 4×10^5 and 2.5×10^6 cpm/0.1 ml of culture fluid by day 9. The amount of RT activity in the cultures treated with a series of granulysin concentrations did not differ from the untreated cultures by more than 20%. Continuous exposure of acutely infected CD4 T cells to granulysin thus did not affect their viability or reduce their ability to replicate HIV.

The results of this study indicate that granulysin does not substantially affect HIV virion infectivity (Table 1), or ongoing virus replication in acutely infected CD4 T cells. The concentrations of granulysin examined in these experiments were sufficient to induce the various

antimicrobial activities reported for this peptide [1,2]. Although the external cell wall or membrane of various Gram-negative and Gram-positive bacteria, fungi, and the parasite *Leishmania* may be sensitive to disruption by granulysin, the envelope of HIV thus appears to be unaffected. The lack of any effect of granulysin on HIV replication in acutely infected cells indicates that granulysin does not prevent spread/reinfection in the cultures. However, because granulysin appears to require the presence of a molecule like perforin to enter the cells [9], these experiments did not address the possible intracellular activity of granulysin – an issue that may not be relevant because HIV does not acquire its envelope until it buds through the outer cell membrane. Therefore, this antimicrobial granule protein probably does not play a direct role in the control of HIV infection.

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Table 1. The effect of granulysin on HIV particle infectivity^a.

| Virus input (TCID ₅₀) | Treatment | RT activity ^b |
|-----------------------------------|------------|--------------------------|
| 3000 | Untreated | 622 ± 41 |
| 3000 | Granulysin | 596 ± 77 |
| 600 | Untreated | 373 ± 11 |
| 600 | Granulysin | 360 ± 10 |

^aEach amount of HIV-1_{SF2} was incubated in diluent (untreated) or 5 mM recombinant granulysin for 3 h at 37°C before inoculating CD4 T cell blasts as described in the text.

^bHIV replication, indicated by the mean ± SD reverse transcriptase (RT) activity ($\times 10^3$ cpm) in 0.5 ml culture fluid at the peak of virus production, is shown. The results are representative of two separate experiments.

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Normalization of the CD4 T cell receptor repertoire after evolution of syncytium-inducing HIV-1 variants

We recently reported on the dynamics of T cell receptor (TCR) diversity during antiretroviral therapy [1]. Interestingly, the TCR diversity of naive (CD45RA+) CD4 cells was as skewed as primed (CD45RO+) cells, which we interpreted as clonal expansions. Earlier it was argued that clonal deletions occur during HIV infection, which lead to perturbations of the TCR repertoire [2,3]. In the setting of viral phenotype switch from non-syncytium-inducing (NSI) to syncytium-inducing (SI) variants, arguments were found against clonal deletion.

For two patients, longitudinal peripheral blood mononuclear cell (PBMC) samples around the NSI/SI switch were fluorescence-activated cell sorter (FACS) sorted in CD4+CD27+CD45RO+ (memory) and CD4+CD27+CD45RO- (naive) subsets. RNA was isolated and 24 TCR-specific polymerase chain reactions (PCR) per sample were performed. PCR products were analysed on size diversity of the TCR complementarity determining region 3 (CDR3) by gel electrophoresis, as described previously [1].

As shown in Fig. 1a, the number of different CDR3 fragment sizes – indicative of TCR diversity – increased during the NSI to SI phenotype switch. The perturbation expressed in percentages, plotted in Fig. 1b, shows a decrease in the number of clonal expansions. At the first timepoint analysed, the patients

showed approximately 40% compared with 10% perturbation in healthy controls. After SI variants had emerged and could be detected (the frequency of cells infected with SI variants were 27 and 175 TCID₅₀/10⁶ CD4 cells for 490 and 1091, respectively), many of the TCR-PCR products showed normalized size distributions. Normalization of the TCR repertoire can either result from a decrease of clonal expansions or from polyclonal replenishment. As the CD4 cell count decreased in both patients, the accompanying normalization of the repertoire may thus result from the disappearance of clonal expansions, allowing lower frequency clones to be detected.

These findings may imply that: first, the naive compartment as defined by CD45RO- CD27+ contains expanded clones. Therefore, at least in HIV infection, CD45 and CD27 markers do not properly define naive CD4 T cells as in CD8 T cells [4]. Second, clonal expansions would seem to be beneficial. As long as patients are able to maintain antigen-driven T helper responses, pathogens are being counteracted. Although the TCR repertoires observed in these patients were improved after the switch, they may actually lack the capacity to mount essential immune responses. SI variants may kill or inhibit activated T cells before they expand, and restricted expansion may result in an accelerated loss of CD4 cells and faster progression to AIDS [5].

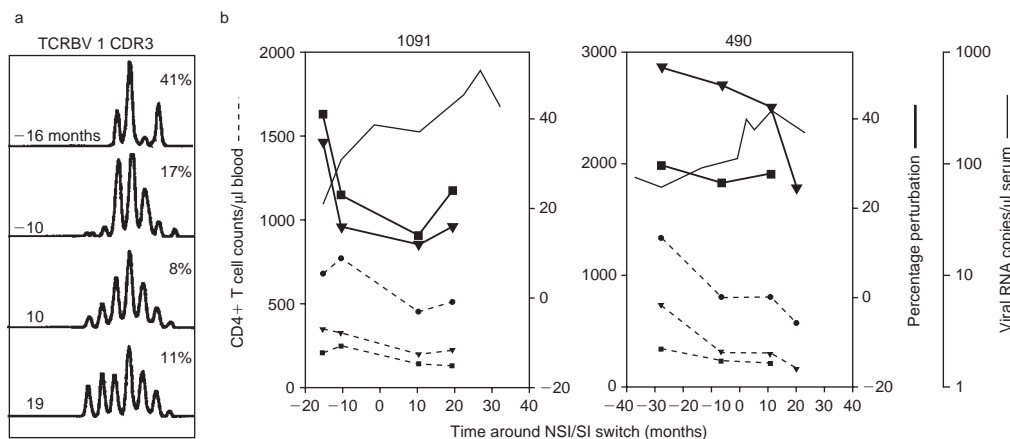


Fig. 1. (a) Polymerase chain reaction products of the varying CDR3 regions of T cell receptor $\text{V}\beta$ (TCRBV) chain. $\text{V}\beta$ family 1 from patient 1091 is shown during HIV phenotype switch from non-syncytium-inducing (NSI) to syncytium-inducing (SI) variants. (b) TCRBV perturbations decrease during viral phenotype switch to SI variants. Naive (\blacktriangledown), memory (\blacksquare), and total CD4 (\bullet) cells decrease in number (dashed lines) as well as in clonality (solid lines, large symbols), whereas the viral RNA plasma load increases (solid thin lines).

Finally, it was observed that seemingly deleted CDR3 sizes reappeared despite ongoing infection. Because there are no reasons to assume that polyclonal thymic T cell renewal is increased, it is most likely that the re-emerging clones and their CDR3 sizes were present before. This means that CDR3 perturbations are not indicative of clonal depletions, as previously suggested [2,3]. It is concluded that perturbations visible in the repertoire reflect previous immune activation rather than immunodeficiency.

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Efavirenz associated with corticosteroids in patients with previous severe hypersensitivity reaction due to nevirapine

Use of the new non-nucleoside reverse transcriptase inhibitors (NNRTI) as part of antiretroviral regimens appears to be an alternative to protease inhibitors (PI) [1–3]. These compounds, especially nevirapine (NVP) and efavirenz (EFV), are potent drugs, which permit more comfortable treatment regimens, and are generally well tolerated. However, they also present some toxicity, hypersensitivity being the most frequent reaction in this class of drugs [1–5]. Little information exists on the possibility of switching from one NNRTI to another, especially in patients who have previously experienced a severe hypersensitivity reaction.

We have recently observed two patients in whom EFV, in addition to corticosteroids, was administered with good tolerance, after they presented with severe hypersensitivity reaction caused by NVP.

Case 1

A 49-year-old HIV-positive woman prostitute with a CD4 cell count of 183 cells/ μ l received several antiretroviral regimens, including nucleosides and PI between August 1996 and October 1998, reaching a CD4 cell count of 589 cells/ μ l and a viral load of less than 500 copies/ml. As a result of intolerance to PI, a regimen including NVP/stavudine (d4T)/lamivudine (3TC) was initiated, causing a severe hypersensitivity reaction with 40°C fever and an intense disseminated and very pruriginous maculopapular rash on day 8 of therapy. Two months later she agreed to receive a regimen with EFV/d4T/3TC in addition to a round of corticosteroids for 11 days (prednisone 30 mg/day for 2

days, 20 mg/day for 3 days, 10 mg/day for 3 days and 5 mg/day for 3 days). She had excellent tolerance to this regimen and in June 1999 her CD4 cell count was 459 cells/ μ l and her viral load was below 50 copies/ml (bDNA 3.0 Chiron).

Case 2

A 38-year-old HIV-infected man, with a history of intravenous opiate use and advanced HIV disease had received different antiretroviral regimens that included zidovudine (ZDV), 3TC, d4T, zalcitabine (ZC) and saquinavir (SQV). He initiated therapy with NVP/d4T/nelfinavir (NFV) in December 1997. At that time, his CD4 cell count was 31 cells/ μ l and his viral load was 415 000 copies/ml. On the seventh day of therapy he presented a severe hypersensitivity reaction consisting of a 40°C temperature and a disseminated, intense and pruriginous maculopapular rash. NVP was stopped and he continued with d4T/3TC/IDV. As a result of virological failure and clinical progression, in May 1999 EFV was administered for compassionate use, forming part of a five-drug regimen (d4T/3TC/RTV/SQV/EFV). Prednisone was added at the same time, the dose being progressively tapering until suspension on day 11 as in patient 1. One week later a mild rash appeared and a new round of steroids was given for 11 days. Since then the patient has continued to receive the same antiretroviral therapy.

This experience suggests that, at least in selected patients with severe hypersensitivity reaction caused by NVP, switching to EFV in association with steroids may be safely carried out, permitting the

continuation of a potent antiretroviral regimen in such patients. As a proportion of patients will not present a cross-hypersensitivity reaction between both drugs [4], it is possible that switching without steroids might also be effective in some cases. Although this might be carried out in patients with mild reactions, it seems prudent to add steroids if a switch is made in patients with severe forms, because fatal hypersensitivity reactions have been described in HIV-infected patients with antiretroviral agents and other types of drugs [6,7].

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Excess peripheral neuropathy in patients treated with hydroxyurea plus didanosine and stavudine for HIV infection

Hydroxyurea potentiates the action of some nucleoside reverse transcriptase inhibitors, and although its role in combination therapy has yet to be fully defined, it is commonly used in combination with didanosine (ddI) and stavudine (d4T). The use of hydroxyurea in HIV infection has recently been reviewed in detail [1]. Although there have been no published reports of peripheral neuropathy in patients receiving hydroxyurea as part of anti-cancer chemotherapy, we have heard anecdotal reports that peripheral neuropathy is commoner in patients taking hydroxyurea as part of antiviral therapy. Neurotoxicity caused by hydroxyurea, presenting with confusion and encephalopathy (but not peripheral neuropathy), has recently been reported in a patient with HIV [2]. We were prompted to examine this issue after observing peripheral neuropathy in five patients receiving ddI, d4T and hydroxyurea in combination with a range of other antiviral agents. In particular, we sought to test the hypothesis that hydroxyurea increases the frequency of peripheral neuropathy in patients receiving ddI and d4T.

Computerized details of antiviral therapy prescribed at the Regional Infectious Diseases Unit are kept within the department. From the records of 938 patients who have been treated in the Unit, 88 treatment episodes were identified in which patients had received ddI and d4T simultaneously. These case notes were examined to determine whether patients had received hydroxyurea and whether they had developed treatment-limiting peripheral neuropathy. Neuropathy was considered to have been caused by antiviral therapy if it had not been

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present before the start of therapy, if the treating physician considered it to be caused by therapy, if therapy had been stopped or changed, and if neuropathy improved after this change or discontinuation.

Hydroxyurea was given in 26 episodes, and seven (27%) of these episodes were complicated by peripheral neuropathy. Didanosine and d4T were given without hydroxyurea in 61 treatment episodes of which six (10%) were complicated by peripheral neuropathy. This difference did not quite achieve statistical significance (Fisher's two-tailed $P = 0.053$). No significant difference was found between the duration of therapy before the diagnosis of neuropathy in either group (Table 1).

Although the increased risk of neuropathy in the patients treated with hydroxyurea did not quite reach a statistically significant level, this is believed to be an important trend that requires further study. Rutschmann *et al*. [3] reported a similar trend towards more frequent and more severe neuropathy in patients receiving hydroxyurea as opposed to placebo with ddI and d4T in a controlled trial of 144 patients (25 and 14%, respectively). Combining these two sets of figures gives an incidence of peripheral neuropathy of all severities of 25.5% for patients on hydroxyurea versus 11.9% for patients not on hydroxyurea (Yates' $\chi^2 = 6.39$, $P = 0.012$).

Thompson *et al*. [4] described a retrospective case-control study in which 23 patients who had received hydroxyurea in association with ddI, d4T, or both,

Table 1. The incidence of peripheral neuropathy in patients receiving didanosine and stavudine with or without hydroxyurea.

| | Hydroxyurea | | No hydroxyurea | |
|---|------------------------|-----------|------------------------|-----------|
| | No PN | PN | No PN | PN |
| n (%) | 19 | 7 (27%) | 55 | 6 (10%) |
| Duration of therapy in days (mean, SD) | 279 ^a (186) | 122 (64) | 317 ^a (278) | 150 (120) |
| Days of therapy to onset of neuropathy (mean, SD) | | 104 (48) | | 90 (95) |
| CD4 cell count at onset of neuropathy (mean, SD) | | 336 (378) | | 263 (290) |

^aTotal duration of treatment, or duration of treatment given up to the date of census (1 August 1999) for patients still on therapy. PN, Peripheral neuropathy.

were compared with 798 patients on similar regimens without hydroxyurea. Five (22%) of the patients exposed to hydroxyurea developed peripheral neuropathy compared with 40 (5%) of patients not exposed to hydroxyurea.

In the past 4 years the number of anti-HIV drugs licensed in Europe has increased rapidly. It is now possible to choose in excess of 100 clinically reasonable three-drug regimens. In a recent survey of 54 patients attending our clinic it was found that 95 different drug combinations had been used (unpublished data). It is clearly no longer possible to assess the efficacy and toxicity of every possible antiviral combination by clinical trial, and it is likely that additive toxicities similar to the one suggested by the data reported in this study will only become apparent with widespread clinical use. In the United Kingdom the Committee on Safety of Medicines, in association with the MRC Clinical Trials Unit and the Medicines Control Agency, have set up an HIV adverse drug reactions reporting scheme, but as of September 1999, no association between neuropathy and hydroxyurea had been reported by that scheme, even though it is believed that this adverse effect is widely recognized by

clinicians. There is clearly a need for clinicians to report adverse effects related to particular antiviral combinations.

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Relationship between Kaposi's sarcoma, Kaposi's sarcoma-associated herpesvirus and AIDS dementia complex

Kaposi's sarcoma (KS)-associated herpesvirus (KSHV) has been discovered by representational differential analysis in AIDS Kaposi's sarcoma [1]. Since the advent of the AIDS epidemic, KS has become one of the most common tumours in parts of Africa, and is the most common tumour found in patients infected with HIV. This virus is clearly associated with all epidemiological forms of KS, and has also been reported with lymphoproliferative disorders such as primary effusion lymphomas and Castleman's disease [2–6].

Although KS has rarely been reported in the brain, KSHV which is strongly linked to KS, could exhibit a tropism for the central nervous system (CNS). Poly-

merase chain reaction base studies have reported the presence of KSHV DNA sequences in sensitive neuronal ganglia from patients with KS [7], in primary lymphomas of the brain [8,9], and in brain samples from patients with multiple sclerosis [10], and KSHV-related encephalitis has been suspected in three patients [11]. However, some of those studies have not been confirmed [12,13], and morphological studies using in-situ hybridization or immunohistochemistry have not yet been performed.

KSHV has been shown to encode chemokine-like proteins (vMIP-I and vMIP-II) [14]. vMIP-II blocks infection of HIV-1 on the surface of cells from a CD4

cell-positive cell line that expresses the chemokine receptor CCR3 for HIV-1 entry into the microglia [15–17]. Because CCR3 is a receptor for HIV-1 entry into the microglia, it was suggested that patients with KS or high loads of KSHV might be less prone to HIV infection of microglia cells, and thus less likely to develop AIDS dementia complex (ADC) [15]. Two previous studies [15,16] have examined the relationship between KS and CNS diseases, including ADC. They demonstrated that patients with KS had a lower frequency of CNS diseases compared with patients who did not have KS during their disease. In a more recent study [18], KS has been identified as a protective effect against ADC.

In order to investigate this hypothesis, a retrospective epidemiological study was conducted on 1960 AIDS patients. Among these patients, four groups were defined: group 1 (KS and ADC; $n = 37$), group 2 (KS without ADC; $n = 738$), group 3 (ADC without KS; $n = 141$) and group 4 (neither KS nor ADC; $n = 1044$).

A chi-square test was performed, which confirmed that patients who develop KS during the course of their AIDS disease had a lower risk of developing ADC ($P = 0.001$).

In order to investigate whether this phenomenon is in relation to KSHV infection or KSHV replication, a retrospective serological study was conducted to compare the rate of KSHV antibodies in the sera from two randomized groups of HIV-infected patients: patients without ADC (group A; $n = 78$) and patients with ADC (group B; $n = 75$) during the course of their AIDS disease. An immunofluorescence assay for antibodies to latent KSHV antigens was performed on a primary effusion lymphoma cell line latently infected with KSHV (BCP1, kindly provided by P.S. Moore and Y. Chang, NY City, USA). This assay was performed as previously described [19]. A KSHV seroprevalence of 17 out of 78 (21.8%) was found in patients from group A, and 15 out of 75 (20%) in patients from group B. The statistical analysis did not show any significant difference between these two groups. However, the geometric mean titre (GMT) of KSHV antibodies was significantly higher in patients without ADC than in patients with ADC, $P = 0.03$, Mann–Whitney non-parametric test (Table 1).

In this study, it was confirmed that patients with KS have a lower risk of developing ADC. The prevalence of KSHV antibodies was similar in patients with and without ADC, suggesting that infection by KSHV does not by itself protect patients from the development of ADC. However, the fact that patients without ADC have a significantly higher GMT of KSHV antibodies than patients with ADC, and the fact that high GMT has been reported to correlate with the presence of KS,

Table 1. Prevalence and titration of Kaposi's sarcoma herpesvirus-specific antibodies by immunofluorescence assay on BCP-1 cells in patients with and without an incidence of an AIDS dementia complex during their AIDS disease course.

| | With ADC HIV-1 ($n = 75$) | Without ADC HIV-1 ($n = 78$) |
|----------|--------------------------------|-----------------------------------|
| KSHV Ab+ | 15 (20%) | 17 (21.8%) |
| Mean | 1/180 | 1/547 |
| Median | 1/100 | 1/200 |
| Max | 1/800 | 1/1600 |
| Min | 1/100 | 1/100 |

ADC, AIDS dementia complex; KSHV Ab+, Kaposi's sarcoma herpesvirus-specific antibodies.

suggests that KSHV active replication could participate in decreasing the incidence of ADC in patients with KS [20]. These results suggest that the lower prevalence of ADC in patients with KS could be related more to KSHV replication than to KSHV infection.

This is in accordance with the fact that vMIP proteins, which have been shown to block CCR3, are mainly produced during the replication phase of KSHV [15].

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Rash as a side-effect of nelfinavir in children

Nelfinavir, formerly AG1343 (Viracept), is a protease inhibitor developed by Agouron and marketed in Europe by Hoffman-La Roche [1]. This is a selective, non-peptidic protease inhibitor of HIV, with a recommended dosage for children of 25–30 mg/kg three times a day (75–90 mg/kg daily). Common reported side-effects include diarrhoea (in 32% of patients) and less frequently rash, nausea, headache and asthenia (5% of patients) [2,3]. Cutaneous side-effects present themselves as frequent problems in the management of HIV-infected patients.

It was observed that 10 out of 42 HIV-infected children treated with nelfinavir presented a typical, self-limited rash. The mean age of the patients (seven girls and three boys) was 73.4 months (range 13–120 months); all them were at different clinical and immunological stages of the disease and nelfinavir was the only common drug used in their combined therapies. The regimens included two nucleoside analogue reverse transcriptase inhibitors (didanosine plus stavudine) in six patients, or stavudine plus nevirapine along with nelfinavir in the remaining patients. Such a treatment was effective in eight out of 10 patients.

The rash started suddenly between days 5 and 9, and the median duration was 8 days. The lesions were erythematous, with generalized maculopapules involving the face, trunk, palms and soles (Fig. 1). These characteristics remained the same independently of the association of nelfinavir to the antiretroviral combinations. A skin biopsy was performed in seven of the



Fig. 1. Erythematous maculopapule lesions on the back.

children, with similar findings in all cases, consisting of changes with basal vacuolation and mild to moderate or intense perivascular infiltration, composed of lymphocytes with variable numbers of eosinophilic leukocytes in the papillary dermis.

The two children who first presented with the rash were critically ill and required polypharmacy. Because of their clinical situation, no antiretroviral drug was withdrawn. The course of the eruption was favourable, and subsequent cases were managed in the same way.

The patients were treated with corticoids and antihistamines (2 mg/kg of prednisone daily and 2 mg/kg of hydroxycine daily) for 3 days. The rash disappeared in 5–7 days and that regimen was stopped. Nelfinavir therapy was not discontinued at any time. Recurrences have not occurred after 12 months of follow-up.

Because nelfinavir was the only common drug taken by all of the patients, and all of them shared the same cutaneous symptoms, it was considered that the rash could be attributed to nelfinavir. This case report outlines the frequency (23.8%) of the rash as a side-effect of nelfinavir therapy, which is higher than that reported so far. Our experience suggests that nelfinavir

should be maintained because of the clinical course of the cutaneous episode. Moreover, the occurrence of the rash has not changed the therapeutic response to nelfinavir and, therefore, this protease inhibitor is still included in the antiretroviral regimen of the reported children.

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Carpal tunnel syndrome in HIV-1 patients: a metabolic consequence of protease inhibitor use?

HIV-1 infection by itself may lead to a number of neurological conditions, prominent among which is a distal sensorimotor polyneuropathy [1,2]. A few short reports have anecdotally suggested that protease inhibitor (PI) use, as part of antiretroviral regimens used to treat AIDS, may rectify this type of neuropathy [3]. No study, however, has reported that any form of neuropathy has resulted as a consequence of such treatment. In this report, four cases of carpal tunnel syndrome (CTS) are presented, which occurred in HIV-1-infected patients and may have been secondary to the metabolic effects of PI use.

Case report

All four patients were women, aged 35–43 years, who had been diagnosed between 1 and 4 years earlier with HIV-1 infection. All presented in June 1999, with at least a 2 month history of either bilateral hand and wrist pain, or complaints of hand swelling and numbness, suggestive of CTS, and were referred for electrophysiological evaluation. CD4 lymphocyte counts ranged from 188 to 836 cells/mm³. All had been treated with PI in combination with reverse transcriptase inhibitors (patients 1 and 4: Viracept; patients 2 and 3: Fortovase) for at least one year, although patient 2 had stopped taking her PI approximately 2 months before being studied because of the development of

lipodystrophy. Two patients (patients 1 and 2) were also taking stavudine (d4T), which has been associated with distal sensory neuropathy in AIDS patients [2] at the time of the studies, although none of the patients reported here had such a condition. Viral loads were undetectable at the time of testing in two cases (patients 1 and 3), just detectable in one case (patient 2) and significantly elevated in another (patient 4). None of these patients were known to have diabetes. Thyroid functions were normal for three patients and unavailable for one.

Standard electrophysiological techniques were used to confirm the diagnosis of CTS. The hallmark of this syndrome involves the selective slowing of median nerve conduction velocities (usually sensory and, to a lesser extent, motor) along the segment of the nerve traversing the so-called carpal tunnel; just distal to the wrist crease [4]. Both arms were studied in all cases.

Median distal motor latencies were prolonged for two patients (bilaterally for patient 1 and on the right for patient 2), and for both those patients the left median motor conduction velocity was mildly slowed (Table 1). The right median sensory response was absent for patient 1. For the remaining wrists, sensory nerve conduction velocities for the segment across the tunnel

Table 1. HIV-1 patients with carpal tunnel syndrome.

| | Patient 1 | Patient 2 | Patient 3 | Patient 4 |
|---|------------|------------|-----------|-----------|
| Age | 41 | 39 | 35 | 43 |
| CD4 cell count (cells/mm ³) | 188 | 520 | 836 | 224 |
| Viral load (virions per ml) | < 50 | 212 | < 50 | 13 745 |
| Protease inhibitor | Viracept | Fortovase | Fortovase | Viracept |
| RTI no. 1 | Zerit | Zerit | Combivir | Combivir |
| RTI no. 2 | Epivir | Epivir | Combivir | Combivir |
| Median nerve – motor | | | | |
| Left | | | | |
| Distal latency (ms) | 4.8 | 4.0 | 3.6 | 3.0 |
| Conduction velocity (m/s) | 45 | 44 | 58 | 51 |
| Response amplitude (mV) | 7.3 | 6.8 | 10.9 | 11.3 |
| Right | | | | |
| Distal latency (ms) | 4.7 | 4.9 | 3.4 | 3.9 |
| Conduction velocity (m/s) | 51 | 50 | 52 | 57 |
| Response amplitude (mV) | 8.4 | 5.4 | 6.5 | 11.4 |
| Median nerve – sensory | | | | |
| Left | | | | |
| Cross-wrist conductance velocity (m/s) | 33 | 26 | 38 | 46 |
| Response amplitude (µV) | 3.5 | 26 | 35 | 42 |
| Right | | | | |
| Cross-wrist conductance velocity (m/s) | NR | 20 | 43 | 44 |
| Response amplitude (µV) | NR | 3.6 | 133 | 33 |
| Patient weights (lb) | | | | |
| 1998 | 159 | 232 | 189 | 102 |
| 1999 | 159 | 223 | 186 | 132 |

NR, Not recorded; RTI, reverse transcriptase inhibitor. Values in bold are abnormal.

were slowed and ranged from 46 down to 20 m/s. Corresponding nerve conduction data for the ulnar nerve were within normal limits, except for mild slowing of the right ulnar sensory conduction velocity for patient 1. All patients thus displayed electrophysiological evidence consistent with mild to moderate CTS.

Numerous risk factors for CTS have been posited, including female sex, repetitive wrist motion, diabetes mellitus, thyroid disease, rheumatoid arthritis and other connective tissue disorders [4–6]. Many authors have noted a significant correlation between CTS and obesity [5–7]. Although all of the current patients were women and at least two were obese, all had also been treated with PI, and it may be that this constitutes an independent risk factor for CTS.

Treatment with PI have important metabolic consequences. Apart from the extensive redistribution of body adipose tissues, a number of laboratory documented abnormalities including hyperlipidemia, insulin resistance, hyperglycemia, decreased glucose tolerance and elevated uric acid levels [8–10] may arise. In one recent study [8], 46% of PI-treated HIV-1 patients developed glucose intolerance and 13% overt diabetes. It may be that some of these effects play a role leading to the development of the focal entrapment of the median nerve across the carpal tunnel. One simple hypothesis might be that PI use in these patients had

led to weight gain and obesity, predisposing them to such nerve entrapment. Indeed, all these patients reported at least a 20 lb weight gain over the previous year, but weights recorded in their clinic records did not bear this out, except for patient 4. Alternatively, it may be that metabolic abnormalities in this context lead to the deposition of myxedematous material in the nerve, as is the postulated mechanism in thyroid disease. Larger controlled trials are clearly necessary to evaluate these hypotheses properly.

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