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Poor penetration of the male genital tract by HIV-1 protease inhibitors

Highly active antiretroviral therapy leads to a reduction in both blood and seminal plasma viral load [1,2]. Some patients on protease inhibitors have, however, demonstrated discordance between viral load suppression in the blood plasma and semen [3]. Genomic differences between blood and semen populations have been documented [4,5], including the demonstration of differences in drug resistance-associated mutations in antiretroviral experienced patients [6].

The male genital tract may therefore represent a distinct 'compartment' in which viral replication and evolution is subject to different selective pressures to those in the blood.

With the recent demonstration of the sexual transmission of a highly drug resistant HIV variant [7] an understanding of these selective pressures becomes important. One determinant will be the concentration of antiviral drugs achieved in this compartment. Poor drug penetration may lead to sub-optimal viral suppression and therefore may predispose to a more rapid emergence of drug-resistant variants.

In this study we have determined the concentrations of saquinavir (SQV) and ritonavir (RTV) in blood plasma and seminal plasma in seven patients receiving triple therapy with RTV, SQV and stavudine (D4T), using validated high performance liquid chromatography [8,9]. The limit of quantification was 25 ng/ml for SQV and 50 ng/ml for RTV.

We tested the hypothesis that matched semen and plasma samples, produced on sequential days at varying specific times after drug ingestion, could produce a drug concentration time curve approximating to those produced by a same day pharmacokinetic (PK) study. The curves created for RTV and SQV in patient 1 are shown in Fig. 1.

This model was then utilized to investigate peak and trough levels of these protease inhibitors in a further six patients receiving RTV, SQV and D4T. The effect of therapy on blood plasma and seminal plasma HIV RNA was assessed by nucleic acid sequence-based amplification (NASBA) [10].

The blood and seminal fluid drug concentrations were measured at steady state. Actual values for the peak and trough levels of each drug are presented in Table 1. Semen drug levels are also expressed as a percentage of the plasma levels. The median seminal plasma/blood plasma peak and trough ratios for RTV were 3.5% (range 1.5–5.2%) and 3.4% (range 0.8–12.9%), respectively. The corresponding values for SQV were 3.6% (0.4–11.3%) and 1.9% (0.7–11.5%), respectively. Median baseline blood plasma and seminal plasma viral loads were 18 000 copies/ml (n = 7) and 4600 copies/ml (n = 6), respectively. By week 24 of therapy, the viral loads for all patients were undetectable in both compartments.

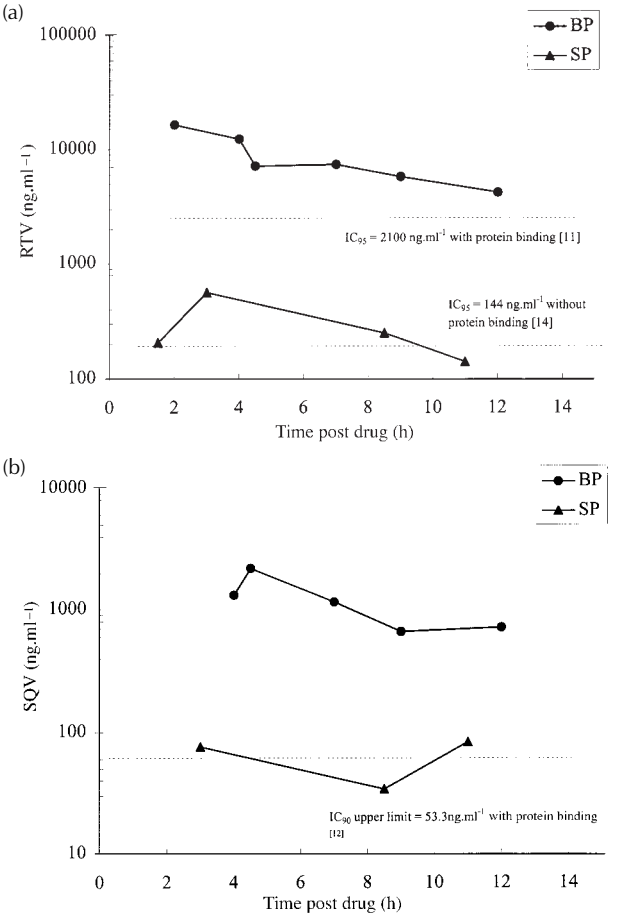

Fig. 1. (a) Plasma concentrations of ritonavir in blood and semen plasma in patient 1 taking ritonavir 400 mg twice a day and saquinavir 400 mg twice a day. (b) Plasma concentrations of saquinavir in blood and semen plasma in patient 1 taking ritonavir 400 mg twice a day and saquinavir 400 mg twice a day.

Table 1. Concentration of ritonavir and saquinavir in blood plasma and semen plasma at peak and trough levels

	RTV peak			RTV trough			SQV peak			SQV trough		
Patient	BP	SP	SP/BP (%)	BP	SP	SP/BP (%)	BP	SP	SP/BP (%)	BP	SP	SP/BP (%)
1	16310	563	4.6	4200	141	3.4	1317	76	5.8	717	83	11.6
2	17390	415	2.4	12510	451	3.6	6260	< 25	0.4	3236	< 25	0.7
3	7140	114	1.6	2800	79	2.8	1904	69	3.6	1295	< 25	1.9
4	13400	670	5	2290	295	12.9	1404	< 25	1.7	319	< 25	7.5
5	15260	790	5.2	10120	870	8.6	742	84	11.3			
6	18020	262	1.5	6020	146	2.4				1307	< 25	1.8
7	12600			6040	< 50	0.8	2187			2430		
Median	15260	489	3.5	6020	146	3.4	1654	69	3.6	1301	< 25	1.9

All ritonavir (RTV) and saquinavir (SQV) levels are ng/ml. Values less than 25 or less than 50 are taken as 24 and 49 for statistical analysis.

If the seminal drug levels achieved are related to the 95% inhibitory concentration (IC_{95}) for both drugs, it can be seen that seminal fluid RTV levels did not exceed the estimated protein adjusted IC_{95} for RTV (2100 ng/ml) [11] at any time point. Similarly, only one of the trough semen SQV concentrations achieved the levels required to exceed the estimated protein corrected IC_{90} values for SQV [12]. Perhaps of more concern with regard to SQV is that some patients had undetectable (< 25 ng/ml) levels of drug in the semen despite extremely high plasma levels.

These data indicate poor penetration of the protease inhibitors RTV and SQV into the semen, with the concentrations achieved being approximately 2–4% of those in blood plasma. It is likely that only free drug (i.e. unbound to protein) is able to access semen. Because semen is highly proteinaceous, however, it is likely that free drug entering the semen will also become highly bound. This has implications for the antiviral effect of these drugs in the male genital tract. Despite this, we have demonstrated good early antiviral efficacy of the combination RTV/SQV/D4T in plasma and seminal fluid. The durability of response will be addressed in ongoing studies. The penetration and efficacy of stavudine within the genital tract obviously requires further study, because this agent has recently demonstrated good penetration into the cerebrospinal fluid.

In conclusion, the poor penetration of antivirals may contribute to a 'compartmental' effect, whereby HIV replication and evolution in the genital tract is subjected to different selective pressures than elsewhere in the body. The observations that drug-resistant mutants are differently represented in the two compartments [13] support this concept. The possibility that viral load and viral species in plasma may not reflect those in the semen should only serve to reinforce the message of safer sexual practices, in order that the transmission of HIV, including drug-resistant variants, is avoided.

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Cytotoxic T lymphocyte recognition of HLA-B*5101-restricted HIV-1 Rev epitope which is naturally processed in HIV-1-infected cells

It is well known that the structural proteins of HIV-1 are effectively recognized by the specific cytotoxic T lymphocyte (CTL) [1]. There are, however, a small number of reports showing Rev-specific CTL activities in HIV-1-infected individuals [2–4] and simian immunodeficiency virus (SIV)-infected macaques [5]. A previous report [6] suggested that an immune response against regulatory proteins provides an efficient defence against viral infection. A potential advantage for the host is that CTL recognition of regulatory proteins enables the destruction of infected cells before the release of infectious virions. Because the HIV-1 Rev is an early regulatory protein [7], it is assumed that the CTL specific for Rev proteins could effectively eliminate HIV-1-infected cells. A recent study showed that HLA-B14-restricted, HIV-1 Rev-specific CTL effectively killed HIV-1-infected target cells [8]. In the present study, we identified an HIV-1 Rev-derived epitope presented by HLA-B*5101, and examined the killing of HIV-1-infected cells by the CTL specific for this epitope.

We have recently identified five HLA-B*5101-restricted CTL epitopes derived from HIV-1 structural proteins by reverse immunogenetics [9]. Similarly, we attempted to identify HIV-1 Rev-derived epitopes presented by HLA-B*5101. Five peptide sequences selected from the Rev protein of the HIV-1 SF2 strain were chemically synthesized. A stabilization assay using RMA-S-B*5101 cells [10] revealed that three (SF2-Rev-75-9:LPPLERLTL, SF2-Rev-69-10:EPV-PLQLPPL, SF2-Rev-71-11:VPLQLPPLERL) of these peptides bound to HLA-B*5101 molecules (data not shown). We attempted to induce the peptide-specific CTL by stimulating peripheral blood mononuclear cells (PBMC) from three HIV-1 seropositive individuals carrying HLA-B51 with these HLA-B*5101 binding peptides. Specific CTL activities for two peptides (SF2-Rev-71-11 and SF2-Rev-75-9) were induced from the PBMC of one HIV-1-infected individual with HLA-B51 after 4 week culture with the corresponding peptides (data not shown), suggesting that these two peptides may be CTL epitopes. To investigate the CTL recognition of these peptides in detail, we generated peptide-specific CTL clones from the bulk culture. The CTL clones for SF2-Rev-71-11 killed C1R cells expressing HLA-B*5101 (C1R-B*5101) pulsed with the corresponding peptides in a peptide concentration-dependent fashion (Fig.1A). These clones showed specific killing of C1R-B*5101 cells infected with a recombinant vaccinia virus containing the HIV-1 SF2 Rev gene (Fig. 1B). In contrast, CTL clones specific for SF2-Rev-75-9 failed to kill the C1R-B*5101 cells infected with the recombinant vaccinia virus (data not shown). These results indicate that SF2-Rev-71-11 is an epitope presented by HLA-B*5101 whereas SF2-Rev-75-9 is not.

Because the SF2-Rev-71-11 epitope (VPLQLP-PLERL) includes the 8-mer peptide VPLQLPPL, both peptides are candidates for the HLA-B*5101-restricted CTL epitope. The SF2-Rev-71-11-specific CTL clone recognized both peptides, but whereas the affinity of the 8-mer peptide for HLA-B*5101 molecules was higher than that of the 11-mer peptide, the concentration of the 8-mer peptide providing the half-maximum lysis was at least 100 times higher than that of the 11-mer peptide (data not shown). These results strongly suggested that the 11-mer peptide is a naturally processed CTL epitope presented by HLA-B*5101 molecules.

It is not known whether SF2-Rev-71-11 can be effectively presented by HLA-B*5101 in HIV-1-infected HLA-B*5101$^+$ cells. We examined the specific killing of the T-B hybrid cell line T1 (HLA-A2$^+$, B51$^+$) infected with HIV-1 SF2 by the SF2-Rev-71-11-specific CTL clones. The ability of two HIV-1 Rev-specific CTL clones (SF2-Rev-71-11-1 and SF2-Rev-71-11-7) and one HIV-1 Env-specific CTL clone (SF2-Env-413-9-335) [9] to kill HIV-1 SF2-infected T1 cells was examined. These CTL clones killed the T1 target cells infected with HIV-1 SF2 but not uninfected T1 target cells, although the killing of the HIV-1 SF2-infected T1 target cells by the Rev-specific CTL clones was weaker than that by SF2-Env-413-9-335 (Fig. 1C). Killing of HIV-1 SF2-infected T1 target cells was blocked by W6/32 anti-HLA class I mAb in a concentration-dependent fashion, whereas it was not inhibited by L243 anti-HLA-DR mAb (data not shown). These results together demonstrate that SF2-Rev-71-11-specific CTL clones recognize the epitope presented by HLA-B51 molecules in HIV-1 SF2-infected cells.

The present study identified an additional HLA-B*5101-restricted HIV-1 epitope. This is also the second HIV-1 Rev epitope that has been precisely analysed. In addition, we demonstrated that HIV-1 Rev-specific CTL can kill target cells infected with HIV-1. The present study thus provides additional evidence that HIV-1 CTL recognizes a naturally processed HIV-1 Rev epitope in HIV-1-infected cells, implying that HIV-1 Rev-specific CTL may eliminate HIV-1-infected cells *in vivo*.

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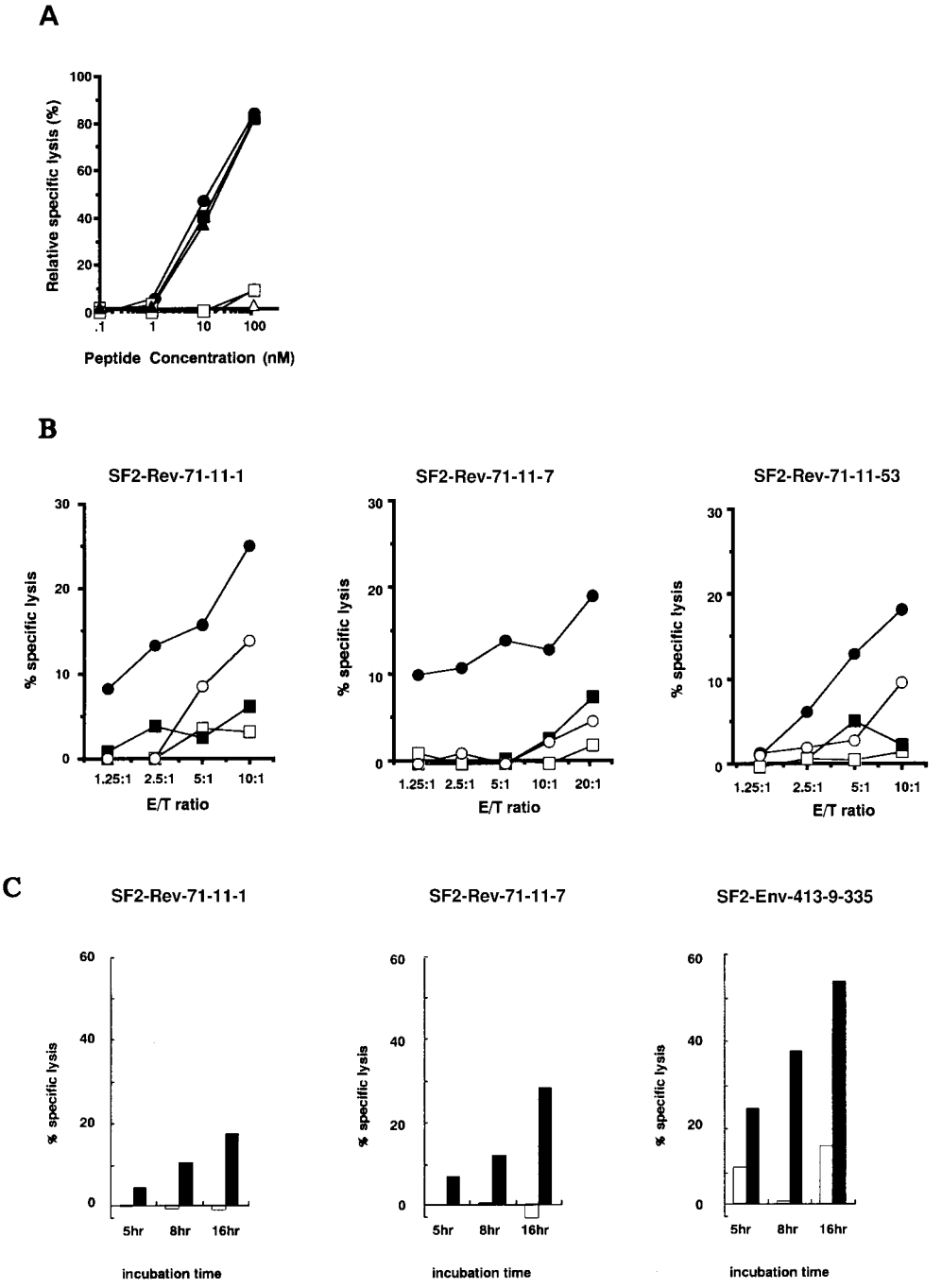

Fig. 1. Recognition of HIV-1 Rev-specific HLA-B*5101-restricted cytotoxic T lymphocyte (CTL) clones. A: The specific CTL activity of three SF2-Rev-71-11 specific CTL clones, SF2-Rev-71-11-1 (●, ○), SF2-Rev-71-11-7 (■, □), and SF2-Rev-71-11-53 (▲, △) was tested at an effector to target ratio of 2:1. C1R-B*5101 cells (●, ■, ▲) and C1R cells (○, □, △) were used as target cells. Cytotoxicity was measured in a standard 4 h ^{51}Cr release assay. The results are shown as relative % specific lysis. (Relative % specific lysis equals % lysis for target cells pulsed with peptide minus % lysis for target cells without peptide). B: Recognition of SF2-Rev-71-11-specific CTL clones for target cells infected with recombinant vaccinia virus encoding the HIV-1 SF2 Rev gene. The recognition of three CTL clones, SF2-Rev-71-11-1, SF2-Rev-71-11-7 and SF2-Rev-71-11-53 was tested in C1R cells and C1R-B*5101 cells infected with either the wild-type vaccinia virus (□, ○, respectively) or the recombinant vaccinia virus (■, ●, respectively). Cytotoxicity was measured in a standard 4 h ^{51}Cr release assay. The results are shown as the specific lysis (%) at various effector and target call ratios. C: The CTL activity of CTL clones SF2-Rev-71-11-1, SF2-Rev-71-11-7 and SF2-Env-413-9-335 for HIV-1 SF2-infected T1 cells (closed bar) and uninfected T1 cells (open bar) was tested by a ^{51}Cr release assay at an effector to target ratio of 5:1. T1 cells were infected with HIV-1 SF2 and were then cultured for 10 days before use as target cells. The CTL activity was measured 5, 8 or 16 h after the addition of the CTL clones.

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Polymorphism at codon 54 of mannose-binding protein gene influences AIDS progression but not HIV infection in exposed children

In the past few years, several studies [1,2] have disclosed a genetic susceptibility to AIDS progression through the analysis of polymorphism of genes encoding for chemokines (as SDF [1]) and their receptor (as CCR5 or CCR2 [2]).

Looking for other genes involved in the genetic susceptibility to AIDS, Garred *et al.* [3] found that homosexual men homozygous for mutant alleles of the mannose-binding lectin (or mannose-binding protein; MBP) gene were at increased risk of HIV infection and of AIDS progression. MBP is an acute-phase protein, secreted by the liver, which participates in the innate immune defence. This lectin may mediate phagocytosis and may activate the classical pathway of the complement [4].

Three different polymorphisms have been described in exon 1 of the MBP gene [5]. Among them, that at codon 54 results in the replacement of a glycine with an aspartic acid and causes a reduction in the level of MBP in the serum of five to 10 times in heterozygous individuals [3].

The present study shows the polymorphism at codon 54 of the MBP gene investigated by amplification-refractory mutation system–polymerase chain reaction (ARMS–PCR) in 52 children with perinatal HIV-1 infection. In this cohort, the time of infection exposure was precisely determined because of vertical transmission at delivery. These patients were divided into two groups depending on their disease progression. The first group included 25 rapid progressor (RP) patients, who developed severe clinical manifestations within the first 2 years of life (category C of the Centers for Disease Control and Prevention (CDC) revised classification system for HIV infection in children); the second group included 27 slow progressors (SP), who had neither progressed to category C nor developed severe immunosuppression beyond 8 years of age. Children with an intermediate disease course were excluded from this analysis. Antiretroviral therapy was given to 19 RP patients and to 16 SP patients, at a mean age of 17.1 (± 16.9) and 59.5 (± 26.2) months, respectively.

Twenty-seven HIV-1-perinatally exposed but uninfected children were also investigated. The HIV-positive and negative children were born to HIV-1-seropositive mothers, who had not undergone any antiretroviral treatment to prevent transmission, because that treatment was not yet in use. No differences were noted between mothers of HIV-infected and uninfected children. Forty-one healthy unrelated subjects matching for ethnic origin served as controls.

Comparing infected and uninfected children born to HIV-1-seropositive mothers, no differences were found in the phenotype frequency of the Gly54Asp allele of the MBP gene. In fact, 18 out of 52 (34.6%) HIV-1-positive children showed the Gly54Asp allele, whereas this allele was present in four out of 27 (14.8%) HIV-1-exposed uninfected children (P = not significant). The

Table 1. Polymorphism at position 54 of mannose-binding protein gene: the frequencies of wild type and mutant alleles were calculated in 52 HIV-1-positive children (subdivided into AIDS rapid (RP) or slow (SP) progressors), 27 exposed-uninfected children and 41 healthy controls

	Exposed infected		Exposed uninfected	Healthy controls
No. of subjects	25 RP	27 SP	27	41
No. of alleles	50	54	54	82
Wild type (%)	37 (74%)*	49 (91%)	49 (91%)	73 (89%)
Gly54asp (%)	13 (26%)†	5 (9%)	5 (9%)	9 (11%)

*Number of alleles (gene frequency %). †RP versus SP: $P = 0.0242$; RP versus exposed-uninfected: $P = 0.0242$; RP versus controls: $P = 0.0247$.

phenotype frequencies of the Gly54Asp mutation of the children were also similar to those of the healthy controls (21.9%) (P = not significant).

The slightly increased frequency of Gly54Asp-positive individuals among HIV-1-infected children was mainly caused by RP patients. In fact, 52% of them tested positive for this variant allele, whereas it was present in only 18.5% of SP patients ($P = 0.011$). Therefore, in HIV-1-infected children, the presence of the Gly54Asp mutation conferred a relative risk of 3.68 (95% confidence limits 1.1–13.1) for a rapid progression to AIDS.

The gene frequency of the Gly54Asp allele was similar in SP children, in exposed uninfected children and in the control population, as expected in the Caucasoid population. Conversely, in RP children, this allele showed a significantly higher frequency than in the other groups (RP versus SP children: $P = 0.0242$; RP versus exposed-uninfected: $P = 0.0242$; RP versus controls: $P = 0.0247$), as shown in Table 1.

These results emphasized that genetic factors predisposing to AIDS should be explored not only in the genes of chemokines or of their receptors, as is mainly done by the majority of current studies, but also in other polymorphic genes coding for proteins playing a role in the immune response.

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Salvage therapy with ritonavir-saquinavir plus two nucleoside reverse transcriptase inhibitors in patients failing with amprenavir–zidovudine–lamivudine

In HIV-positive patients, virological failure of protease inhibitors (PIs) plus nucleoside reverse transcriptase inhibitors (NRTIs) is frequent. The efficacy of second-line PI-regimens has been evaluated in patients failing with currently available PIs. Amprenavir is a new PI showing, both *in vitro* and *in vivo*, little genotypic and phenotypic cross-resistance with other PIs [1,2]. Clinical data concerning the efficacy of second-line PI regimens in patients failing with amprenavir are currently lacking.

We retrospectively reviewed six Caucasian homobisexual males, who failed virologically with amprenavir–zidovudine (ZDV)–lamivudine (3TC) and subsequently underwent a second-line PI regimen. Their median age was 39 years (range 29–47). Failure with amprenavir plus ZDV–3TC was defined as either 1 $\log_{10}$ increase of viral load (Amplicor™) from nadir (patients 1, 2, 4, 6) or return of viral load to baseline (patients 3, 5). Median baseline viral load and CD4 cells were 4.6 $\log_{10}$ copies/ml and 358 cells/μl, respectively; baseline Centers for Disease Control and Prevention (CDC) category was B in all patients but patients 1 (A) and 4 (C). Patients were given ritonavir (600 or 400 mg twice a day) (patients 1, 3, 5) plus saquinavir (400 mg twice a day) and two NRTIs: stavudine–didanosine (d4T–ddI) in five patients either naïve to

Table 1. Viral load and CD4 cell response

Patient		1	2	3	4	5	6
Viral load log_{10}	BL	5.20	4.19	4.13	4.61	5.88	4.51
	Ml	ND	< 2.60	< 2.60	3.10	ND	< 2.60
	M3	2.80	< 2.60	4.12	3.43	5.13	< 2.60
	M6	2.95	< 2.60	< 2.60	4.19	5.87	< 2.60
	M9	2.96	< 2.60	5.49	4.56	5.81	< 2.60
CD4 cells/rnm^3	BL	375	399	457	342	208	331
	Ml	501	336	704	628	ND	349
	M3	ND	509	575	697	149	471
	M6	695	570	837	686	194	378
	M9	418	658	365	618	276	464

BL, baseline; ND, not done.

both (patients 1, 3, 5, 6) or at least to ddI (patient 2), or d4T–3TC in one patient experienced to both (patient 4). The mean follow-up was 12.4 months (range 10.5–14.2). After 9 months, three patients were responders, with viral loads either below 400 copies/ml (patients 2, 6) or decreases of 2 log_{10} at least from baseline (patient 1), one patient virologically responded and relapsed twice (patient 3), and two patients were failures, with viral loads either returned to baseline (patient 4) or not changed from baseline (patient 5). All but patient 3 improved their CD4 cell count (Table 1). No AIDS-defining events or death occurred. Tolerance was good, one patient (patient 6) required the substitution of d4T by ZDV for severe paresthesia. Two patients temporarily discontinued their treatment for personal reasons (patient 3 from months 2 to 3 and from months 7 to 9; patient 5 from months 2 to 4).

In conclusion, after a 9 month follow-up, viral suppression was obtained in three out of four compliant patients and immune response in five out of six patients. These data suggest that ritonavir–saquinavir rescue could be effective in amprenavir-pretreated patients, in contrast with the short-term efficacy found in saquinavir, ritonavir or indinavir experienced patients [3]. Further studies in larger cohorts of patients are warranted to confirm these data, which could reflect the low cross-resistance between amprenavir and other PIs [1,2].

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Lipodystrophy associated with nevirapine-containing antiretroviral therapies

HIV protease inhibitors (PIs) used in antiretroviral combination therapy have been increasingly implicated in reports of lipodystrophy, a phenomenon associated with truncal adiposity and peripheral fat wasting [1–4]. The morphological changes in patients treated with PIs have also included lipomatosis, in which fatty deposits are found in the supraclavicular fossae and across the shoulders resulting in a 'buffalo hump' appearance [5,6] as well as breast enlargement [7]. A mechanism explaining the pathogenesis of PI-associated lipodystrophy has recently been proposed [8], although it remains unclear whether this hypothesis explains similar cases reported in other HIV treatment groups [9,10].

The association of lipodystrophy with HIV PIs has prompted a re-evaluation of combination therapies in favour of PI-sparing regimes, which might avoid the development of this side-effect.

In this short report we describe a series of patients developing lipodystrophy on a PI-sparing regime containing nevirapine (Viramune, Boehringer Ingelheim, Germany), a non-nucleoside reverse transcriptase inhibitor, which is commonly used as a component of highly active antiretroviral therapy (HAART).

To evaluate the prevalence and pattern of nevirapine-associated lipodystrophy, we reviewed the records of PI-naïve patients who had taken a nevirapine-containing HAART regime (nevirapine plus two nucleoside analogues) for at least 6 months. Fifty-eight evaluable patients satisfied these criteria. Lipodystrophy was

Table 1. Clinical features of patients with lipodystrophy

Subject	Sex	Age	Peripheral fat loss	Prominent peripheral veins	Central obesity	Facial thinning	Breast enlargement	Weight change (kg)*	Baseline viral load (copies HIV-1 RNA/ml)	Baseline CD4 ($\times 10^9$/l cells)	Antiretroviral therapy	Duration of therapy before onset of lipodystrophy (months)
1	Male	54	–	–	+	+	–	–5.8	151 785	0.272	d4T/3TC/NVP	15
2	Male	35	+	+	+	–	–	1.2	471 911	0.004	ZDV/3TC/NVP	7
3	Male	41	+	+	+	–	–	–0.9	8673	0.335	d4T/3TC/NVP	21
4	Male	54	+	+	+	–	–	0.5	8000	0.162	d4T/ddI/NVP	9
5	Male	48	+	–	–	–	–	–4.9	1200	0.463	d4T/3TC/NVP	13
6	Female	54	–	–	–	–	+	1.5	125 980	0.230	d4T/ddI/NVP	7
7	Female	38	–	–	+	–	–	+9	20 689	0.363	d4T/ddI/NVP	22
8	Female	37	+	+	+	–	+	+1.7	NR	0.402	ZDV/3TC/NVP	8
9	Male	31	+	+	+	–	–	+1.4	125 992	0.200	d4T/ddI/NVP	14

NR, Not recorded; –, no marked change; +, clinically obvious; *weight change (+ gain, – loss) recorded from start of treatment to onset of lipodystrophy. NVP, nevirapine; d4T, stavudine; 3TC, lamivudine; ZDV, zidovudine; ddI, didanosine.

assessed by patients self-reporting or clinic staff noting changes in body shape confirmed by clinical observation. Nine patients (16%) had clinical features in keeping with the lipodystrophy syndrome (Table 1), with central obesity being the most commonly reported feature. Breast enlargement was a characteristic confined to two of the three female patients. None of these nine patients had an AIDS-defining illness before starting treatment, and none developed AIDS during nevirapine-HAART. For eight of the nine patients, the mean baseline plasma viral load was 114 278 copies HIV-1 RNA/ml (HIV Amplicor, Roche Diagnostic Systems, Somerville, New Jersey, USA). The mean baseline peripheral blood CD4 cell count for all patients was 0.270×10^9/l cells (TruCount, Becton–Dickinson Immunochemistry Systems, San Jose, California, USA).

In all cases, the development of lipodystrophy was associated with an undetectable viral load (< 400 copies HIV-1 RNA/ml). At the onset of lipodystrophy, the mean rise in peripheral blood CD4 cell count was 0.236×10^9/l, with a range of $0.036–0.629 \times 10^9$/l cells. The mean time to the onset of lipodystrophy from the start of nevirapine-HAART was 12.9 months.

Our findings suggest that lipodystrophy occurs in patients on nevirapine-containing HAART regimes, and lends support to the notion that this syndrome is not a unique feature of HIV PI treatment. Although the prevalence of nevirapine-associated lipodystrophy has not previously been reported, our rate of 16% appears to be comparable with a prevalence of 13% seen with PI-associated lipodystrophy reported by Shaw *et al.* [4], but was not as high as that initially reported by Carr *et al.* [2] (64%).

It has been suggested that the pathogenesis of PI-associated lipodystrophy may be caused by these agents binding to and altering the function of human proteins involved in lipid metabolism or insulin signalling [8], although there appears to be some dispute over the exact mechanism by which PIs exert their influence *in vivo* [11]. These hypothetical mechanisms, however, do not explain the same or very similar phenomena seen in this and other reports [9,10], in which lipodystrophy has occurred without PIs being used. There appears to be a consistent association with lipodystrophy occurring in patients showing an optimal response to antiretroviral therapy, as reflected in the marked reduction in the plasma concentration of HIV-1 viral RNA below the limit of detection. This suggests that, rather than lipodystrophy being an adverse effect specific to PI therapy, its development may indicate a relief of metabolic stress in some patients consequent on potent retroviral suppression.

This report serves to highlight the observation that lipodystrophy is not a phenomenon specific to HIV PI therapy. Accordingly, physicians and their patients who may have considered the option of PI-sparing antiretroviral drug regimes as a way of avoiding lipodystrophy should be alert to the possibility of its occurrence as a general consequence of effective antiretroviral therapy.

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Cytomegalovirus (re)activation plays no role in the ocular vitritis observed after initiation of highly active antiretroviral therapy

Cytomegalovirus (CMV) retinitis occurs in almost one third of patients with AIDS and is associated with severe immune depression [1]. Highly active antiretroviral therapy (HAART) induces at least partial immune restoration and improves the long-term outcome of CMV retinitis [2,3]. Early after the institution of HAART, however, a relapse of CMV retinitis often presenting as vitritis has been reported [4,5]. Similarly, an initial worsening of hepatitis B and C co-infections and mycobacterial infections followed by a beneficial long-term outcome in most cases has been reported after the start of HAART [6]. Immune recovery was documented to be the leading cause in these infections. For CMV retinitis after HAART, the same mechanism was hypothesized, but to date has not been proven [4,5,7]. In this report, we document a patient in whom the mechanism of the HAART-associated CMV retinitis/vitritis was examined in the intraocular fluid obtained by anterior eye chamber paracentesis.

The patient is a 37-year-old homosexual man with AIDS and very low CD4 cell counts (10×10^6/l), who developed severe bilateral CMV retinitis. He was successfully treated with a 3 week combination drug regimen of intravenous ganciclovir and foscarnet. During maintenance therapy with these drugs for more than 1 year, the retinitis relapsed once unilaterally, necessitating a second course of induction therapy. After HIV protease inhibitors became available, the patient's two-drug anti-HIV therapy of zidovudine and zalcitabine was switched to a triple combination of stavudine, lamivudine and indinavir. Within 6 weeks, the CD4 cell numbers rose from 20 to 320×10^6/l. Concurrently, a relapse of the CMV retinitis was diagnosed by fundoscopic examination. Because of associated vitritis, an anterior eye chamber paracentesis was performed. The ratio of circulating antibody titre with that inside the eye fluid, the so-called Witmer–Goldmann coefficient, was determined. The intraocular fluid was also examined by polymerase chain reaction assays. Meanwhile, induction therapy with ganciclovir and foscarnet was started. The diagnostic tests of the eye chamber fluid did not reveal any activity of CMV or any other known ocular pathogen (other herpes viruses or *Toxoplasma gondii*). Consequently, anti-CMV therapy was stopped without sequelae.

In our patient, an infectious cause of the retinitis was excluded while he responded to HAART with an exponential initial increase of CD4 cells. From these findings, immune restoration appears to be the most plausible causative mechanism of the HAART-associated retinitis/vitritis. Our findings fully support the former hypothesis that relapses of CMV retinitis after the initiation of HAART were caused by the HAART-induced initial immune response [4,5].

The final approach to this so-called immune recovery vitritis remains to be established. Anti-CMV therapy seems not to be particularly helpful. Some cases might benefit from immune suppressive therapy with local corticosteroids, although most cases recover spontaneously [5,7].

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Gemfibrozil effectively lowers protease inhibitor-associated hypertriglyceridemia in HIV-1-positive patients

HIV protease inhibitors have become the foundation for combination antiretroviral chemotherapy, have reduced the mortality and morbidity of advanced HIV disease [1], and are now widely used. Peripheral lipodystrophy has been reported among patients receiving protease inhibitors, and hypertriglyceridemia has been reported with ritonavir [2].

The long-term risks of hypertriglyceridemia in patients not infected with HIV include accelerated atherosclerosis and pancreatitis, which have been reduced by triglyceride-lowering treatment in population-based studies [3]. Two cases of severe premature coronary artery disease were reported in HIV-infected men in association with protease inhibitor therapy [4].

Gemfibrozil lowers serum triglycerides in patients with dyslipidemia [5]. We reviewed our experience with gemfibrozil to determine its effectiveness in lowering serum triglyceride elevations associated with HIV-1 protease inhibitors.

A database of 672 HIV-positive patients in an urban HIV clinic identified 14 patients treated with gemfibrozil. Of these, nine were prescribed gemfibrozil after protease inhibitor therapy and eight patients received gemfibrozil for at least 4 weeks. A retrospective review of these eight medical records was conducted. Demographics, antiretroviral therapy and laboratory values were recorded. Serum triglycerides were evaluated before the use of HIV-1 protease inhibitors, during protease inhibitor therapy and during concomitant protease inhibitor and gemfibrozil therapy. It was not possible to determine whether or not the serum triglyceride level was fasting. Patients were judged to be adherent to gemfibrozil if the record indicated regular refills of their prescription, or if clinical staff documentation of patient self-reported adherence existed.

A total of eight men received gemfibrozil 600 mg orally twice a day. Three were Caucasian, two were African-American, two were Hispanic and one was a Native American. At the start of gemfibrozil treatment, the median age of the patients was 47 years, the CD4 cell count was 152 cells/mm^3 and the viral load was 13 200 copies/ml. The patients (n) received in recommended doses: ritonavir [4], nelfinavir [2], indinavir [1] and ritonavir/saquinavir combination therapy [1].

The patients received gemfibrozil for a median of 175 days (range 76–376). Serum triglycerides increased from a median of 298 (158–661) to 1803 (716–2847) mg/dl after 223 (41–394) days of protease inhibitor therapy. The median nadir serum triglyceride level after gemfibrozil was 300 (173–490) mg/dl after 89 (37–255) days, a reduction of 83% (Fig. 1). The median serum triglyceride level at 20 ± 4 weeks (n = 6) was 417, a 77% reduction. Serum total cholesterol ranged between 200 and 230 mg/dl and did not change with gemfibrozil treatment.

One patient, who after an initial response, became non-adherent with gemfibrozil, and developed a marked rebound in serum triglyceride level, responded again once adherence improved. There were no adverse reactions attributed to gemfibrozil during its administration.

Hypertriglyceridemia was reported in the AIDS before the clinical use of HIV protease inhibitors [6–8]. A presumed association with cytokines and immune activation was not definitively established. Hypertriglyceridemia has been reported with ritonavir [2]. Ritonavir was the protease inhibitor used in five of our eight patients (one in combination with saquinavir), but nelfinavir and indinavir were associated with the remaining three cases. Therefore, hypertriglyceridemia appears to be a possible adverse experience with any current HIV protease inhibitor.

A mechanism by which protease inhibitor-associated hypertriglyceridemia may occur was recently proposed [9]. The active site of the HIV-1 protease has a sequence homology of 60% with two human proteins that regulate lipid metabolism: cytoplasmic retinoic-acid binding protein type 1 (CRABP-1) and low density lipoprotein-receptor-related protein (LRP). CRABP-1 produces cis-9-retinoic acid, which binds to retinoid X receptor, which in turn forms a dimer with peroxisome-proliferator activated receptor (PPAR) type gamma to stimulate adipocyte differentiation and inhibit lipocyte apoptosis. LRP stimulates hepatic chylomicron uptake and triglyceride clearance. CRABP-1 or LRP bound with protease inhibitor would result in hypertriglyceridemia.

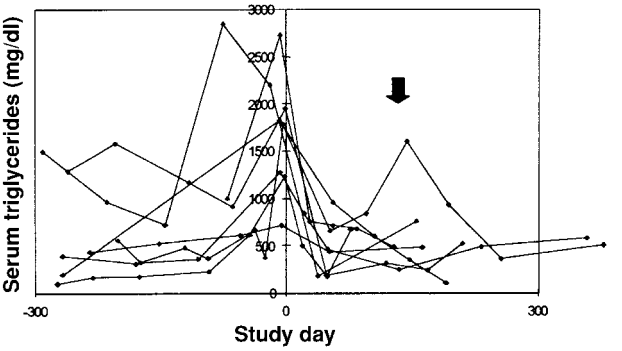

Fig. 1. Serum triglyceride levels in eight patients before and during protease inhibitor administration (negative study days) and with the co-administration of gemfibrozil (positive study days). Note the elevation and subsequent decline in one patient who became temporarily non-adherent with gemfibrozil (arrow).

Gemfibrozil reduces serum triglycerides in HIV-uninfected persons, and is indicated in the treatment of a variety of dyslipidemias. Gemfibrozil inhibits peripheral lipolysis, decreases the hepatic extraction of free fatty acids, reduces hepatic triglyceride production, and inhibits the synthesis while decreasing the clearance of very low density lipoprotein apo B [5]. The molecular mechanism of action of gemfibrozil had not been defined when it was approved for clinical use. The class of fibrates to which gemfibrozil belongs is, however, believed to stimulate PPAR type alpha [10]. The stimulation of PPAR type alpha, which promotes the oxidation of fatty acids while inhibiting *de novo* fatty acid synthesis, could compensate for the reduction in PPAR type gamma to influence serum triglyceride levels.

The time course and long-term risk of untreated protease inhibitor-associated hypertriglyceridemia is unknown. Protease inhibitor-associated lipodystrophy, of which hypertriglyceridemia is one manifestation, does not appear to remit over time with continued protease use. As a result, it is likely that affected patients will require lipid-lowering therapy for an extended period of time.

Gemfibrozil appeared to reduce serum triglyceride levels effectively in adult male HIV-1-positive patients with protease inhibitor-associated hypertriglyceridemia. This represents a preliminary, uncontrolled observation that should be confirmed with prospective, randomized trials.

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Reply to Gonzalez and Everall: Lest we forget: neuropsychiatry and the new generation anti-HIV drugs

In their review about neuropsychiatric disorders and the new generation of anti-HIV drugs, Gonzalez and Everall [1] refrain from mentioning the neuropsychiatric side-effects of efavirenz. These central nervous system (CNS) symptoms occur relatively frequently and include: dizziness, headache, confusion, stupor, agitation, amnesia, depersonalization, euphoria, hallucinations, insomnia, abnormal dreaming, anxiety, depression and suicide ideation [2,3].

CNS symptoms may appear after the first dose and may resolve spontaneously after a few days or weeks. Certain minor CNS symptoms, such as dizziness, can be avoided by taking the drug at bedtime. Patients who complain of nightmares while taking the usual 600 mg dose at bedtime can be advised to switch to a twice a day regimen.

In the presence of serious neuropsychiatric side-effects such as anxiety, depression and suicide ideation switching to another antiretroviral drug may be needed.

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High plasma levels of nelfinavir and efavirenz in two HIV-positive patients with hepatic disease

Efavirenz (EFV) is a novel non-nucleoside reverse transcriptase inhibitor (NNRTI) with potent anti-HIV activity that has been shown to increase (by approximately 20%) the plasma levels of nelfinavir (NFV) in volunteeers [1]. Many interactions between EFV and NFV have still to be studied, particularly in patients with liver disease, because the two drugs share a common metabolic pathway through cytochrome P450. When evaluating the pharmacokinetic (PK) profile of NFV and EFV in a cohort of patients treated with a combination of EFV, NFV and stavudine (d4T), we recently saw two cases of very high plasma levels of NFV and EFV in individuals with different degrees of hepatic dysfunction.

Case 1

M.E., a 35-year-old homosexual man who had been HIV positive since 1992. Episodes of bacterial pneumonia and *Pneumocystis carinii* pneumonia (PCP) were present in his medical history; hepatitis B and C infections were excluded. Past antiretroviral treatments included zidovudine (ZDV) plus didanosine (ddI) and d4T, lamivudine (3TC), ritonavir (RTV) (interrupted for virological failure). In December 1997, he was shifted to a combination of d4T, NFV and EFV. Before starting this regimen he had an HIV plasma viral load of 336 000 copies/ml, a CD4 cell count of 87 cells/μl and slightly elevated aminotransferase levels (aspartate aminotransferase (AST) 41 mU/ml, alanine aminotransferase (ALT) 49 mU/ml reference normal value: up to 40 mU/ml) but with normal gamma glutamyl transferase (γ-GT) levels. In June 1998, AST and ALT levels were 163 and 128 mU/ml, respectively, whereas γ-GT was 1621 mU/ml. A liver ultrasound showed an enlarged liver. The abnormal liver function tests were attributed to drug-induced hepatitis. He reported confusion and dizziness, which was interpreted as an EFV-related side-effect. Concurrent medications included cotrimoxazole and azithromycin. EFV and NFV PK samplings were done in May 1998.

Case 2

M.S., a 39-year-old female ex-intravenous drug abuser (IVDA), who had been HIV positive since 1989, had a history of PCP, cytomegalovirus (CMV) retinitis and hepatitis C virus (HCV)-related liver cirrhosis. She had been treated with different drug combinations including ZDV, ddI, hard gel (HG)-saquinavir (SQV) (discontinued because of decompensated cirrhosis) and d4T, 3TC, indinavir (IDV) (interrupted because of kidney stones and virological failure). In March 1998, with a HIV-1 plasma viral load value of 272 000 copies/ml and a CD4 cell count of 60 cells/μl, she was started on d4T, NFV and EFV. At that time AST and ALT levels were 223 and 149 mU/ml, respectively. Just after having started the rescue combination, she complained about NFV-associated diarrhoea. The HCV-RNA level was 23×10^6 copies/ml. An upper gastrointestinal (GI) endoscopy revealed the presence of gastritis and liver ultrasonography showed a small, irregularly shaped, hyperechogenic liver. Concomitant medications included pyrimetamine, furosemide, ganciclovir and aerosolized pentamidine isethionate. In June 1998 she underwent PK sampling.

Pharmacokinetic data

Blood samples for PK evaluation of the two drugs were obtained at steady-state conditions, at different times during the dosing interval. NFV and EFV plasma concentrations were quantified by specific high performance liquid chromatography (HPLC) assays [2,3].

The results are displayed in Table 1, where the figures obtained in the two patients with the elevation of liver enzymes caused by different causes are compared with the means from our own cohort (as this is an ongoing investigation we pooled data from 11 patients for EFV and from 18 patients for NFV). There was a striking elevation of total body systemic exposure to both drugs in both patients. The area under the curve (AUC) and the average plasma concentration (Css) during the dosing interval increased by 288 and 363% for EFV in patients 1 and 2, respectively, whereas the corresponding increases were 168 and 154% for NFV. The PK values obtained from this control cohort compared well with those in the literature: the EFV PK profile determined in volunteers [1] yielded a mean AUC_{0-24} of 78.1 μg/h/ml and a mean Css of 3.2 μg/ml, whereas no data are so far available for HIV-positive individuals. The NFV PK values also agreed well with those reported in a recent paper [4].

In HIV-positive patients elevated liver function tests may be caused by hepatotropic viruses, opportunistic infections, alcohol or drug abuse and other conditions [5]. Highly active antiretroviral therapy (HAART) may worsen the liver damage already existing in HCV–HIV

Table 1. Pharmacokinetic data of the two patients compared with a control cohort

	EFV Css	EFV AUC_{0-24}	NFV Css	NFV AUC_{0-8}
Patient no. 1	9.7	232.5	5.9	47.3
Patient no. 2	11.5	277.1	5.6	44.8
Controls*	2.5 (1.7–3.5)	59.8 (41.5–83.1)	2.21 (0.45–3.6)	17.7 (3.6–36)

Concentration at steady state (Css) expressed as μg/ml. Area under the curve during a dose interval (AUC) expressed as μg/h/ml. *Data obtained from the control cohort (see text). For efavirenz (EFV) median value from 11 subjects; for nelfinavir (NFV) from 18 patients (range in brackets).

co-infected patients, possibly as a result of an enhancement of HCV replication [6]. It is also likely that an altered metabolism (and then increased plasma levels) of drugs capable of inducing liver damage, may play a further role in this setting [7]. Despite the usually large variability of PK parameters in HIV-positive individuals, the significant increase in NFV and EFV systemic exposure found in two patients with high aminotransferase values (three to four times the normal values), should be carefully evaluated, even though no adverse events of unexpected intensity were observed. If other data were to confirm this finding, individual EFV and NFV dosage adjustments in HIV-positive patients with hepatic damage could be implemented in the same manner as has already been suggested for other antiretroviral agents such as RTV [8].

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Analysis of the CC chemokine receptor 5 m303 mutation in infants born to HIV-1-seropositive mothers

The CC chemokine receptor 5 (CCR5) functions as the major co-receptor for macrophage-tropic strains of HIV-l [1]. Several polymorphisms identified within the coding and the promoter regions of the CCR5 gene have been found to affect HIV-1 infection and disease progression. One of these, a 32 base pair deletion (Δ32) in the CCR5 coding region, results in a truncated protein that is not expressed on the cell membrane [1] . The Δ32 genetic variant affords protection against infection by macrophage-tropic strains in individuals who are homozygous for this allele, and partial protection against disease progression in heterozygotes [1]. Moreover, a T→A point mutation that introduces a premature stop codon at position 303 (m303) of the CCR5 coding region prevents functional co-receptor expression; m303 heterozygosity combined with the Δ32 deletion on the other allele confers resistance to HIV-1 infection [2]. Other uncommon genetic variants of the CCR5 coding region have been described, but their relevance to HIV-1 co-receptor function has not been completely established [3–5]. A large number of studies demonstrated that the frequency of the Δ32 allele varied according to the geographical area, and was approximately 10% in Caucasian populations [6]. Only two studies have addressed the m303 variant to date; one reported heterozygosity for this allele in one of 18 HIV-l-exposed individuals, and in three of 209 healthy blood donors of unknown ethnic origin [2]; in the other, the m303 allele was detected in one of 35 African Americans at high risk of HlV-1 infection [4].

As both the m303 mutation and the Δ32 deletion impair CCR5 co-receptor function, we analysed the two genetic alterations in a cohort of 397 infants (90 HIV-1-infected and 307 uninfected) born to HIV-1-seropositive mothers.

After Δ32 analysis by polymerase chain reaction (PCR) as previously reported [7], nine infected and 27 uninfected infants were found to be heterozygous for this allele; Δ32 homozygosity was detected only in two uninfected children. These results agree with the reported frequency of the Δ32 allele in southern Europe, and confirm that Δ32 heterozygosity in itself does not protect against vertical transmission [8]. m303 analysis was performed by PCR, using 5′-GGT GGAACAAGATGGATTATCAAGTGT-3′ (5′–3′: –11–16) and 5′-CAGCATGGACGACAGCCAGG-3′ (3′–5′: 400–381) as forward and reverse primers, respectively [9], followed by the digestion of amplification products with HincII restriction enzyme. Only one uninfected child, without the Δ32 deletion, was

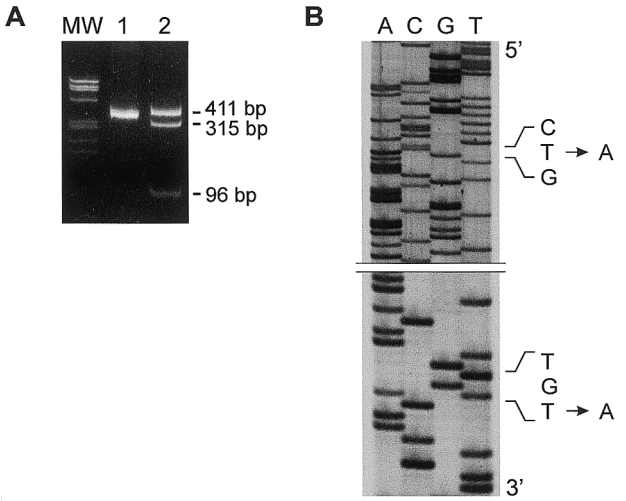

Fig. 1. Search for the CCR5 m303 mutation discloses a heterozygous allele in an HIV-1-uninfected infant. A. Amplification product (lane 1) was digested with *Hin*cII restriction enzyme (lane 2) and electrophoresed on a 4% polyacrylamide gel. After *Hin*cII digestion, the wild-type fragment was cleaved into 315 and 96 base pair fragments; the mutated fragment was not cleaved because of the loss of the *Hin*cII restriction enzyme. MW, molecular weight marker ϕx 174/Hae III. B. Sequence analysis of the same PCR amplified DNA, using a T7 Sequenase Kit version 2.0 and the antisense primer located at nucleotide position 3′–5′:400–381 of the CCR5 coding region, revealed a heterozygous T→A mutation at nucleotide position 303, and a homozygous T→A mutation at position 164.

found to be heterozygous for the m303 allele (Fig. 1, panel A). This finding was confirmed by the sequencing of PCR amplified DNA (Fig. 1, panel B). Sequence analysis was performed using a T7 Sequenase Kit version 2.0 (Amersham Life Science, Cleveland, OH, USA) and the reverse primer employed in the PCR analysis. It is interesting that sequence analysis disclosed that the infant carrying the m303 allele was homozygous for the T→A point mutation at nucleotide position 164; this finding was confirmed by sequence analysis of two separate amplified products. This non-silent mutation creates a leucine/glutamine amino acid change within the putative first transmembrane domain of the CCR5 receptor, and does not seem to affect HIV-1 co-receptor function [5]. Like m303, this allelic variant is also found at low frequency, varying from 0.04 to 0.07 according to the ethnic group [4].

Our findings confirmed the presence of the m303 allele in an Italian cohort of 307 HIV-1-uninfected children born to HIV-1-seropositive mothers with an allele frequency of 0.002, and did not reveal m303 alleles among 90 HIV-l-infected children. The finding of two uncommon genetic alterations, m303 and 164A, in the same individual could suggest a linkage disequilibrium of the two alleles. In this regard it would be of interest to search for the 164A mutation in the individuals reported to carry the m303 allele.

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