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The correlation between levels of IL-7Rα expression and responsiveness to IL-7 is lost in CD4 lymphocytes from HIV-infected patients

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Measurements of Bcl-2 and CD25 expression suggested that IL-7R function is modified in CD4 lymphocytes of untreated viraemic patients. The extent of IL-7R function restoration post-HAART was analysed. A positive linear relationship was demonstrated between IL-7Rα expression and the magnitude of IL-7-induced responses in healthy individuals, whereas this relationship is lost in HIV-infected patients, suggesting that viraemic patients suffer a receptor signaling transduction defect in IL-7R function. IL-7 responsiveness is only partly restored by HAART.

IL-7 is a well-known lymphopoietic cytokine that plays a critical role in peripheral T-lymphocyte homeostasis. IL-7 is required both for the survival of naive T-cell populations and the homeostatic cycling of naive and memory cells [1]. Plasma levels of IL-7 are increased in HIV-infected patients [2]. These elevated levels may affect the CD4 cell count, and we have previously shown that HIV-infected patients with low IL-7 levels before the start of HAART have a diminished capacity for immune reconstitution [3]. However, this rise in IL-7 levels does not prevent the CD4 cell count from falling during HIV infection. In addition, HIV infection may involve defects in the IL-7/IL-7R system. A decrease in IL-7Rα (CD127) expression was first reported to occur in CD8 T cells from HIV-infected patients [4]. A similar trend was more recently reported for CD4 T cells [5]. In addition, a further reduction in IL-7Rα expression in CD4 T cells was observed in individuals with advanced HIV disease. More recently, we demonstrated that IL-7R function is impaired in CD4 lymphocytes from untreated chronically HIV-infected patients, as measured by the reduced IL-7 induction of both the anti-apoptotic molecule Bcl-2 and the activation marker CD25 [6]. In this report we focus our attention on whether or not the changes noted in the IL-7 responsiveness of CD4 T cells from HIV-infected patients are related to reduced levels of CD127 chain expression.

The relationship between CD127, Bcl-2 and CD25 expression was first examined in a control group of healthy individuals (Fig. 1). Mean fluorescence intensity (MFI) values for CD127-positive CD4 T cells were used to assess the level of CD127 expression. It was seen that surface levels of CD127 expression varied within the group (MFI median and quartiles values 162.8; 151.7–192.7). By contrast, the vast majority of CD4 T cells in the group were CD127 positive, and percentages of CD127-positive CD4 cells were very similar from one individual to another (96.7%; 96.1–97.3). We followed the IL-7-induced expression of Bcl-2 and CD25 by measuring the relative increases in their MFI in CD4 T cells stimulated *in vitro*. Spearman rank correlation coefficients showed that CD127 expression levels in healthy individuals were linearly correlated with IL-7-induced Bcl-2 and CD25 expression ($r = 0.82$, $P < 0.004$, $r = 0.68$, $P < 0.03$, respectively). This is therefore the first study to identify a positive linear association between the levels of IL-7Rα on the surface of CD4 T cells and the amplitude of the response induced by IL-7 in healthy individuals. Under normal physiological conditions, this feature may contribute to the mechanism underlying the biological effects of IL-7 because increasing the levels of CD127 would continuously modulate the magnitude of the IL-7 response. Moreover, under our experimental conditions, there is no evidence that the surface level of CD127 determines a threshold of reactivity in CD4 T cells. Consequently, the surface levels of CD127 can be predictive of IL-7 responses in healthy individuals.

We then further analysed the data obtained from two groups of HIV patients previously described [6]. The group of untreated chronically infected patients was characterized by a plasma viral load of 17 100 RNA copies/ml (8900–26 700) and CD4 cell counts of 401 cells/μl (286–740) at enrollment. Briefly, as reported before, we noted in the patients a slightly lower percentage of CD127-positive CD4 cell percentage (88.3%; 87.2–89.4) and somewhat lower CD127 cell surface expression (MFI 95.7; 91.6–104.0) than in the healthy controls. A striking reduction was observed in IL-7-induced Bcl-2 and CD25 expression. The relative increase in Bcl-2 was reduced to 15.2% (10.3–20.0) of the values noted in the controls. The reduction in IL-7-induced CD25 expression was more moderate (54.9%; 45.4–63.5). Here we failed to detect any significant correlation between CD127 levels and the IL-7-induced responses in this group (Fig. 1), indicating that surface levels of CD127 did not account directly for the reduced IL-7 responses. Furthermore, in this group, the levels of IL-7-induced Bcl-2 and CD25 expression were clearly correlated ($r = 0.75$, $P < 0.02$ Spearman's test), indicating that the decrease in Bcl-2 and CD25 expression in this group had a similar source. IL-7 plasma levels were higher

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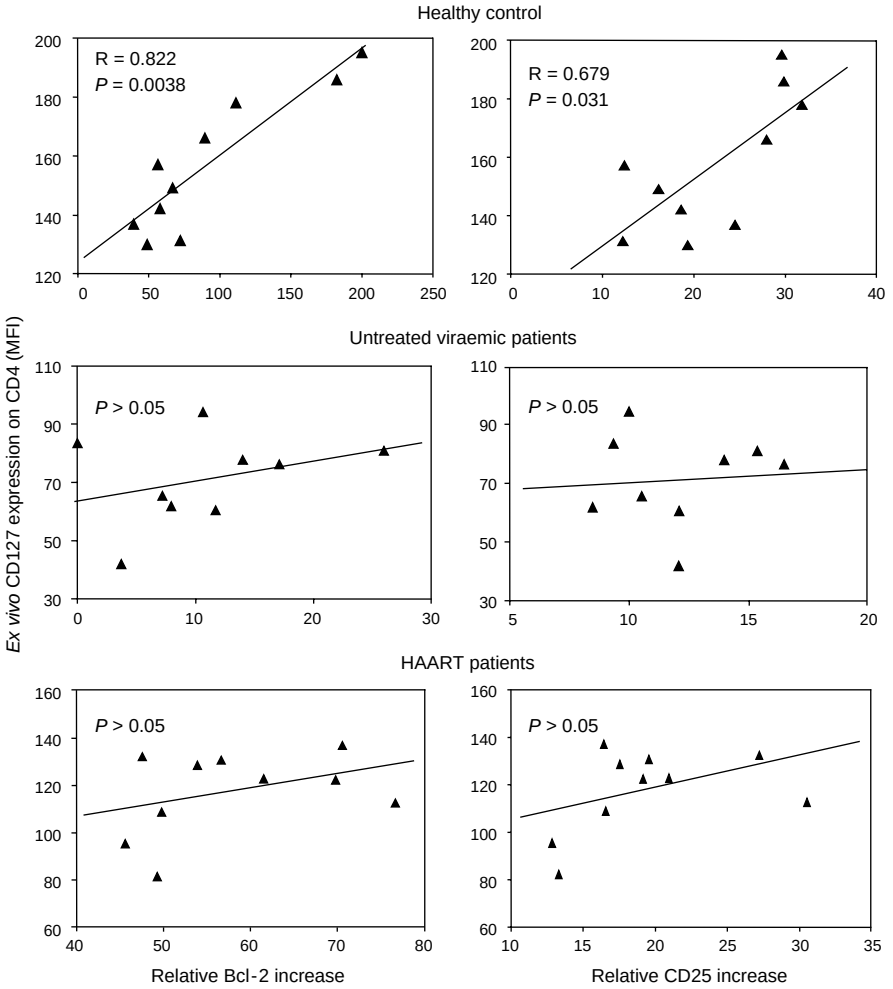

Fig. 1. Correlation between levels of IL-7Rα expression and IL-7-induced Bcl-2 and CD25 expression in CD4 cells from untreated HIV-infected and HAART patients. Two groups of chronically HIV-1-infected patients were included in this study (Clinical Trials EP20 and EP-33 conducted by the Agence Nationale de Recherche sur le Sida) $N = 10$ for each group. The first group consisted of untreated viraemic patients with a plasma viral load greater than 10 000 RNA copies/ml and a CD4 cell count greater than 350 cells/μl at inclusion. The second group included patients who had been receiving HAART for at least one year, with CD4 cell counts greater than 400 cells/μl. Blood samples from healthy donors ($N = 10$) were used as controls and were obtained from the Blood Transfusion Center at the Percy Hospital in Clamart, France. CD127 expression was evaluated on peripheral blood mononuclear cells immediately after Ficoll separation. Mean fluorescence intensity (MFI) values obtained in CD4 CD127-positive cells are used to represent the levels of CD127. MFI values for the entire CD4 cell subset were used to evaluate Bcl-2 and CD25 levels. Measurements of IL-7-dependent Bcl-2 and CD25 induction were obtained by subtracting the values obtained after 6 days of culture in the absence of IL-7 from those obtained in the presence of IL-7. The expression of CD4, CD25 and CD127 were analysed with anti-CD4-activated protein C (anti-CD4-APC), anti-CD25-FITC or anti-CD127-R-phycoerythrin monoclonal antibodies. To measure Bcl-2 expression, cells were first labeled with anti-CD4-APC before permeabilization and staining by anti-Bcl-2-FITC monoclonal antibodies. Correlation coefficients and P values were calculated by Spearman's test.

in the untreated group (16.2 pg/ml; 8.3–21.2) than in the control group (1.4 pg/ml). Given that IL-7 has the capacity to downregulate the expression of its own receptor [7], we investigated a possible correlation between the levels of CD127 expression and levels of IL-7 in the plasma. No statistically significant correlation was found (Spearman rank correlation test $P > 0.05$) making it unlikely that levels of circulating IL-7 have a direct effect on this parameter.

The data obtained from the group of patients who had been given HAART for at least one year were also analysed. Here the viral load was under control at 50 copies/ml plasma or less over the previous 6 months and immune restoration was evident with CD4 cell counts of 526 cells/μl (486–597). A slight decrease in the CD127-positive CD4 percentage (95.7%; 93.1–96.4) and in CD127 MFI (132.4; 113.0–141.2) was nevertheless observed. IL-7 stimulation *in vitro* increased Bcl-2

and CD25 (79.0%; 70.4–96.8) and 83.5% (74.9–93.7), respectively, relative to healthy controls. This is consistent with the fact that CD4 lymphocytes from HAART patients are more IL-7-responsive than those from untreated viraemic patients (see above). However, as shown (Fig. 1), the correlation between the surface levels of CD127 and IL-7-induced Bcl-2 expression was not restored, and the same was observed for CD25 expression. This indicates that the capacity of IL-7R to modulate CD4 T-cell responsiveness to IL-7, as seen in the controls, was not recovered in the HAART group.

Taken together, our results provide further insights into the mechanism by which T-cell homeostasis is disrupted during HIV disease. Our data obtained in untreated viraemic patients suggest that behind the reduction in CD127 expression, downstream of IL-7R, cellular defects may contribute to a decrease in IL-7 responsiveness. This defect may involve part of the IL-7R signal transduction pathway such as the Akt/phosphatidylinositol-3 kinase or the JaK/STAT pathway [8]. The effect may also be related to cellular defects independent of the IL-7/IL-7R system. This would be in agreement with our previous work describing abnormalities in the IL-2 response of T lymphocytes from HIV-infected individuals [9,10], which were found to be associated with dysfunction of the JaK/STAT pathway, which is shared by the IL-2R and IL-7R systems [8]. We have previously described that HAART partly restores IL-7-induced Bcl-2 and CD25 expression [6]. The data presented here further suggest that altered IL-7 responsiveness somehow persists in HAART patients. This may have profound consequences on the partial immune response restoration generally observed with HAART, and should be taken into consideration when evaluating the therapeutic potential of IL-7.

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Virological characterization of an infection with a dual-tropic, multidrug-resistant HIV-1 and further evolution on antiretroviral therapy

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We studied a case of recent infection with multidrug-resistant (MDR) HIV-1. Over 16 months off-therapy, the CD4 cell count decreased from 419 to 184 cells/μl. Antiretroviral therapy (ART) then led to an incomplete virological response but to an immunological benefit, concurrently with a shift to CCR5-only tropism and a reduction in replication capacity. ART, even if suboptimal, can be of interest in the case of MDR virus infection.

Multidrug-resistant (MDR) HIV-1 variants with resistance to three classes of drugs (nucleoside and non-nucleoside reverse transcriptase inhibitors, and protease inhibitors) can

be transmitted [1–3], although infrequently [4–6], and present unique challenges for the treatment of HIV in such individuals. Here, we report one case of MDR HIV-1 transmission originating from a cohort of recent seroconverters in south-western France and associated with rapid immunodeficiency. We particularly emphasize the evolution of the virological characteristics of the transmitted variant.

The patient was enrolled in the Aquitaine cohort [7]. A retrospective analysis of the prevalence of drug-resistant variants was performed within this cohort. Patients were included if they had a reliably estimated date of HIV-1 seroconversion between 1996 and 2005, either with laboratory evidence of acute infection, or a previously seronegative test within 3 years. In this latter case, the estimated date of seroconversion was the mid-point between the last negative and the first positive serology. Genotypic resistance analysis was processed on the first available plasma sample within 18 months of the estimated date of seroconversion and before the initiation of antiretroviral therapy (ART). Resistance mutations were reported as listed by the International AIDS Society – USA Panel (www.iasusa.org, updated October 2005) and the ANRS algorithm version 2005 (www.hivfrenchresistance.org), excluding minor protease mutations and the V118I reverse transcriptase (RT) mutation. For selected plasma samples, phenotypic drug susceptibility, replication capacity assays and HIV tropism assays (Monogram Biosciences, South San Francisco, California, USA) were performed as previously described [8,9]. In case of MDR HIV-1, the host HLA typing was determined by sequence specific primers polymerase chain reaction, and the CCR5 gene was genotyped by nucleotide sequence analysis.

The RT and protease sequences were determined for 229 patients who seroconverted between 1996 and 2005. The prevalence of transmitted resistance was 15.7% (95% confidence interval 11.0–20.4%), considering resistance or possible resistance as defined by the ANRS algorithm. One of the 229 patients (0.4%) was shown to be infected by a virus with resistant mutations to three classes of drugs. The patient was a 27-year-old gay man with a negative HIV serology in August 2000 and a complete HIV-1 Western blot in November 2001, with an estimated date of seroconversion of April 2001. The patient was asymptomatic, Centers for Disease Control and Prevention stage A, at entry. The first genotype was performed in December 2001 before receipt of ART, and detected resistance mutations to three classes of drugs (Table 1). The ANRS algorithm predicted resistance or possible resistance to all drugs, except lamivudine and emtricitabine. No genotypic resistance to enfuvirtide was found by gp41 sequence analysis. The virus belonged to HIV-1 subtype B.

The patient was followed over the ensuing 16 months on no ART and had a rapid CD4 cell count decline from 419 to 184 cells/μl during this period. The genotype was repeated at 17 months post-seroconversion, still off-ART and showed conservation of most protease and RT resistance mutations. At this time, the phenotypic tests showed resistance to all RT and protease inhibitors, a replication capacity that was comparable to the wild-type reference isolate, and a dual/mixed tropism.

At 24 months post-seroconversion the patient began ART, with lamivudine/tenofovir/lopinavir/ritonavir, switching to lamivudine/tenofovir/atazanavir/ritonavir 13 months later, because of the gastrointestinal side effects of the lopinavir/ritonavir combination, and with the knowledge of the resistance tests results. In the setting of ART, a partial control of HIV-1 replication was achieved, with an immunological benefit, with CD4 cell counts sustained over 200 cells/μl. Genotypic characterization was performed on lamivudine/tenofovir/atazanavir/ritonavir after 28 weeks on ART, showing minimal genotypic evolution but the additional selection of the RT M184V mutation. At this time the phenotypic testing showed resistance to all ARV but tenofovir, a decrease in replication capacity to 36%, and a shift to CCR5-only tropism (Table 1). This CCR5 tropism was confirmed on a subsequent sample drawn at week 57 postseroconversion, while the patient remained asymptomatic. The patient's human leukocyte antigen genotype showed no evidence of human leukocyte antigen alleles associated with disease progression. The sequence of the CCR5 gene showed no mutation or deletion.

We characterized a case of a recent infection with a MDR HIV-1 leading to a rapid decrease in the CD4 cell count in the absence of treatment. This patient was the only case of triple-class multidrug resistance from a cohort of 229 ART-naive patients infected between 1996 and 2005. The patient's isolate was characterized before receipt of ART and showed a replication capacity comparable to wild-type and dual/mixed tropism, both of which may be associated with rapid disease progression [10,11]. These characteristics are similar to the case described by Markowitz *et al.* [2]. Both cases describe a conjunction of distinct viral characteristics, determined by different viral genes and associated with higher potential pathogenicity. The clinical context is, however, different because our patient did not report recreational drug use, contrary to the New York case. The phylogenetic analysis of recent infections from the Aquitaine cohort did not reveal any cluster related to this case, suggesting limited transmission in this population. The subsequent virological and immunological evolution on therapy showed a partial control of viral replication despite resistance to most of the antiretroviral drugs of the regimen, the patient remaining clinically asymptomatic. However, at last available follow-up, the patient is being treated by a less than optimal regimen, and he remains under close clinical surveillance.

Table 1. Virological and immunological evolution and viral characteristics before and during antiretroviral therapy.

Time post SC (months)	ART	Viral load	CD4 cell count	Genotype and phenotype	% RC (CI)	Tropism
8	–	6064	419	Genotype: NRTI: M41L, E44D, D67N, V75T, L210W, T215Y, K219N NNRTI: K101E, Y181C, G190S PI: L10I, K20R, M36I, I54V, L63P, A71V, G73T, I84V, L90M	nd	nd
17	–	11262	294	Genotype: NRTI: M41L, E44D, D67N, V75T, L210W, T215**H**, K219N NNRTI: K101E, Y181C, G190S PI: L10I, K20R, M36I, I54V, L63P, A71V, G73T, I84V, L90M Phenotype: NRTI: ABC: 3.76; ddI: 1.95; FTC: 6.05; 3TC: 5.04; D4T: 2.09; ZDV: 3.52; TDF: 1.44 NNRI: EFV: >MAX; NVP: >MAX PI: ATV: 169; fosAPV: 14; IDV: 57; LPV: 50; NFV: 92; SQV: >MAX; TPV: 22	103% (65–163)	R5/X4
24	Started on 3TC/TDF/LPV/R	23149	184	nd	nd	nd
37	Switch to 3TC/TDF/ATV/R	1210	302	nd	nd	nd
52	3TC/TDF/ATV/R	742	175	Genotype: NRTI: M41L, E44D, D67N, V75T, **M184V**, L210W, T215**Y**, K219N NNRTI: K101E, Y181C, G190G/S PI: L10I, K20R, M36I, **F53F/L**, I54V, L63P, A71V, G73T, I84V, L90M Phenotype: NRTI: ABC: 11; ddI: 2.91; FTC: >MAX; 3TC: >MAX; D4T: 2.67; ZDV: 6.66; TDF: 1.21 NNRI: EFV: 146; NVP: >MAX PI: ATV: >MAX; fosAPV: 21; IDV: 82; LPV: 66; NFV: 134; SQV: >MAX; TPV: 24	36% (23–57)	R5
57	3TC/TDF/ATV/R	10192	304	nd	nd	R5

ABC, abacavir; ART, antiretroviral therapy; ATV, atazanavir; CI, confidence interval; ddI, didanosine; D4T, stavudine; EFV, efavirenz; fosAPV, fosamprenavir; FTC, emtricitabine; IDV, indinavir; LPV, lopinavir; >MAX, value over the maximum detection limit; nd, not determined; NFV, nelfinavir; NNRTI, non-nucleotide reverse transcriptase inhibitor; NRTI, nucleotide reverse transcriptase inhibitor; NVP, nevirapine; PI, protease inhibitor; /R, boosted by ritonavir; RC, replication capacity; SQV, saquinavir; TDF, tenofovir disoproxilfumarate; TPV, tipranavir; 3TC, lamivudine; ZDV, zidovudine.
Genotype: additional mutations appear in bold. Phenotypic results are expressed as sensitivity index reported to the wild-type PNL4-3 strain.

The relative benefit of this incomplete viral suppression could be caused by the decrease in viral replication capacity (probably driven by the additional selection of M184V), by residual drug activity, and by the shift to CCR5-only tropism. The mechanism of this shift in tropism to R5 virus merits some discussion. One possibility is the presence of a mixed viral population at baseline consistent with an HIV co-infection or super-infection [12]. The tropism determination as dual/mixed tropic is not inconsistent with this possibility. In this setting ART might have been more effective for a subpopulation of X4 viruses. It is also possible that negative immune selection against the CXCR4-utilizing populations may be relevant in this case of early HIV infection.

In conclusion, we described a case of infection by an MDR, dual/mixed tropic HIV-1, leading to a rapid decrease in CD4 cells. A potentially inactive ART and a switch to R5-only tropism were then associated with an immunological improvement, suggesting that therapeutic intervention can be of interest even in this virological context. New classes of antiretroviral drugs are, however, needed to better control viral replication of MDR viruses.

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Appendix

Composition of the Groupe d'Epidémiologie Clinique du SIDA en Aquitaine (GECSA) – ANRS CO3 Aquitaine cohort:

Scientific committee: Prs J. Beylot, M. Dupon, M. Longy-Boursier, J.L. Pellegrin, J.M. Ragnaud and R. Salamon (Chair). Scientific coordination: Prs F. Dabis and G. Chêne, Medical coordination: Drs N. Bernard, D. Lacoste, D. Malvy, D. Neau, Prs M. Dupon, J-F. Moreau, P. Morlat, P. Mercié, J.L. Pellegrin and J.M. Ragnaud. Biostatistic coordination: D. Commenges, H. Jacqmin-Gadda, R. Thiébaut. Clinical studies monitor: Dr S. Lawson-Ayayi. Data manager: E. Balestre. Technical team: M.J. Blaizeau, M. Decoin, S. Delveaux, C. Hannapier (CISIH), S. Labarerre, V. Lavignolle-Aurillac and B. Uwamaliya-Nziyumvira (INSERM U.593), G. Palmer and D. Touchard (CREDIM, ISPED), D. Dutoit, F. Pereira and B. Boulant (CREDIM, ISPED). Participating hospital departments (participating physicians): Bordeaux University Hospital: Pr J. Beylot (Pr P. Morlat, Drs N. Bernard, M. Bonarek, F. Bonnet, B. Coadou, P. Gelie, D. Jaubert, C. Nouts, D. Lacoste), Pr P. Couzigou (Dr L. Castera), Pr H. Fleury (Pr M.E. Lafon, Drs B. Masquelier, I. Pellegrin et P. Trimoulet), Pr M. Dupon (Drs H. Dutronc, G. Cipriano, S. Lafarie, C. De La Taille, Pr. J.Y. Lacut), Pr J.L. Pellegrin (Drs J.F. Viallard, C. Cipriano, I. Faure, P. Rispal, S. Tchamgoue, Pr B. Leng), Pr M. Longy-Boursier (Pr P. Mercié, Drs D. Malvy, T. Pistone, P. Thibaut, R. Vatan, J.P. Pivetaud, Pr M. Le Bras), Pr J.F. Moreau (Dr J.L. Taupin), Pr J.M. Ragnaud (Drs C. De La Taille, D. Neau, T. Galperine, S. Lafarie, D. Chambon et A. Ochoa), Pr C. Series, Pr A. Taytard; Dax Hospital: Dr M. Loste (Drs L. Caunègre et F. Fonade); Bayonne Hospital: Dr F. Bonnal (Drs S. Farbos, M. Ferrand et M.C. Gemain); Libourne Hospital: Dr J. Ceccaldi.

Hair versus plasma concentrations as indicator of indinavir exposure in HIV-1-infected patients treated with indinavir/ritonavir combination

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Large intra-individual variability in plasma levels may limit the interest of therapeutic drug monitoring based on a single determination. Indinavir concentrations were determined both in plasma and hair samples, and correlated with concomitant plasma HIV-RNA in 43 HIV-infected patients. In multivariate analysis, significant association was found between HIV-RNA below 50 copies/ml and indinavir concentrations in hair but not in plasma, suggesting that hair concentrations gave more extensive information on drug exposure than a single plasma sample.

In HIV-infected patients, the rapid achievement of an undetectable viral load, which is noted in less than 70% of patients, has been linked to a long-term favourable

clinical prognosis [1,2]. It is thus necessary to increase as far as possible the rate of early virological success. Improvements in adherence have proved to be of benefit in some patients [3]. Because of the level of high interpatient variability and the efficacy–concentration and toxicity–concentration relationships, the optimization of protease inhibitor doses based on plasma concentrations could also be beneficial [4–6]. However, the benefit of therapeutic drug monitoring is under debate. A single plasma concentration interpretation in the context of common practice is often difficult, because of the high inter and intrapatient variability of pharmacokinetics, in the patient's notification of time intervals between the last drug intake and sampling, and in treatment adherence and co-medications [7]. We showed that in non-fully adherent, protease inhibitor-naive patients receiving indinavir, the efficacy–concentration relationship disappeared when the adherence score of each patient was analysed, whereas it was maintained in fully adherent patients [8]. This is probably explained by the fact that the plasma concentration is the indicator of individual antiretroviral pharmacokinetic characteristics with day-to-day variability, but also of adherence history during the days preceding blood sampling. Furthermore, as a result of the high intra-individual variability in plasma concentrations even in fully adherent patients, adaptation of the protease inhibitor dose has the highest chance of being effective if based on several concentration determinations, at best over several days thus creating a patient concentration 'profile' [7]. However, repeated blood sampling is difficult to obtain in everyday life, and may delay necessary interventions. As indinavir accumulates gradually over time in the hair, the determination of indinavir concentrations in the hair, reflecting a longitudinal intake and therefore less susceptible to day-to-day variability, could allow an easier and more accurate determination of exposure and analysis of the efficacy–concentration relationship. It has already been shown in patients receiving indinavir 800 mg three times a day that indinavir hair concentrations were higher in patients with undetectable viral loads than in those without [9,10]. However, the concomitant indinavir plasma level was not monitored in those studies; therefore information generated by plasma measurement and hair measurement could not be compared. We thus studied in a transactional study the relationship between virological success and the indinavir hair concentration, plasma concentrations, and other factors usually associated with virological success [1].

Indinavir-boosted ritonavir-receiving HIV-infected patients treated for more than 4 months were included in this study. Proximal hair was cut close to the scalp and crushed to a powder; hair concentrations were expressed as μg/g of hair, as previously published [10]. Indinavir trough plasma concentrations before the morning drug intake and hair concentrations were measured in each patient by a validated high-performance liquid chromatography method (limits of quantification: 5 ng/ml and 0.5 μg/ml, respectively) [11]. For each patient, daily and cumulative (since the initiation of the indinavir-boosted ritonavir-containing HAART) indinavir doses were calculated; patients with concomitant HIV-RNA levels below 50 copies/ml were classified as being virological responders. Univariate and multivariate analyses of associated factors were performed using a logistic regression model in which virological success was the primary outcome. Odds ratios with 95% confidence intervals (CI) were estimated.

Of the 43 patients included, 29 (67%) were classified as being virological responders. All patients were receiving low-dose ritonavir (100 mg twice a day), 31 patients were taking indinavir 400 mg twice a day, six patients were taking indinavir 200 mg twice a day, and the six other patients were taking 600 mg twice a day. The median indinavir concentration in the hair was 10 μg/g [interquartile range (IQR) 5–15 μg/g] and the median trough plasma indinavir concentration was 390 ng/ml (IQR 212–675 ng/ml).

Median indinavir concentrations in the hair were 15 μg/g (IQR 6–21 μg/g) in virological responders and 8 μg/g (4–11 μg/g) in non-responders (Fig. 1), whereas the median plasma indinavir trough concentration was 370 ng/ml (IQR 210–680) in virilogical responders

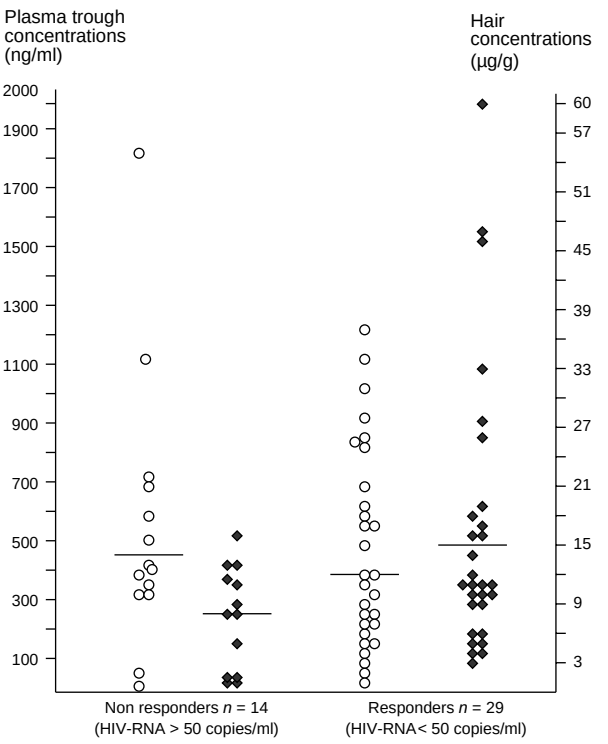

Fig. 1. Indinavir plasma trough and hair concentrations in virological responders (HIV-RNA < 50 copies/ml) and non-responders (HIV-RNA > 50 copies/ml) in HIV-infected patients receiving indinavir/ritonavir-containing HAART. ○ Plasma trough concentrations; ◆ hair concentrations.

and 480 ng/ml (IQR 212–675 ng/ml) in non-responders. Neither indinavir concentrations in the hair, nor trough indinavir concentrations in plasma were correlated with indinavir daily doses. In multivariate analysis, the indinavir hair concentration remained the only factor associated with virological success [$P = 0.04$; odds ratio (OR) 3.88; 95% CI 1.01–14.94], whereas sex (male versus female; $P = 0.06$; OR 5.91; 95% CI 0.90–38.73), baseline protease inhibitor-naive patient status ($P = 0.29$; OR 5.22; 95% CI 0.23–115.93), high baseline HIV-RNA level ($P = 0.06$; OR 0.68; 95% CI 0.47–1.01), and indinavir trough plasma concentration ($P = 0.13$; OR 0.99; 95% CI 0.99–1.01) were not.

As already found for patients receiving non-boosted indinavir, hair concentrations were related to virological success; the concentrations observed in virological responders and non-responders were in the same range as those previously observed in patients with non-boosted indinavir [10]. Furthermore, when also analysing the concomitant plasma concentration and other virological success-associated factors, we found that the determination of the indinavir concentration in hair was more accurate for predicting virological success than a single determination of the indinavir plasma concentration. As we have previously found in analysing the part played respectively by adherence and pharmacokinetic characteristics in the concentration–efficacy relationship, a single plasma concentration determination without concomitant data on adherence within the previous day leads to a misinterpretation of the results [8]. It is particularly striking in the case of the four virological non-responder patients without detected resistance-associated mutations for whom indinavir plasma levels were accurate whereas the indinavir concentrations in hair were low, probably revealing a poor adherence level. Monitoring the indinavir plasma concentration in the hair allows a longitudinal assessment of indinavir impregnation in a large time window, which takes into account the individual pharmacokinetic characteristics, including intraindividual variability, and the patient's adherence history [7]. It has recently been shown in highly adherent patients receiving protease inhibitors with sustained undetectable viral loads in whom extensive repeated plasma levels were measured that the intra-individual coefficient of variability was up to 45%, which may limit the utility of a single measurement in therapeutic drug monitoring for protease inhibitors [7].

Therefore, in the context of therapeutic drug monitoring, ritonavir-boosted indinavir hair sampling may be a useful tool to monitor indinavir impregnation and to help interpret concomitant plasma concentrations allowing an adequate decision based on a same day determination. This combined procedure represents a less traumatic means of patient data collection avoiding repeated plasma sampling and delays in clinical interventions.

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Zinc fights diarrhoea in HIV-1-infected children: in-vitro evidence to link clinical data and pathophysiological mechanism

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Diarrhoea-related morbidity is reduced by zinc supplementation in HIV-1-infected children. The mechanisms of this effect are largely undefined. We

provide evidence for role for Tat (transactivating peptide produced by HIV-1) in the pathogenesis of diarrhoea in AIDS patients. In this study we showed that zinc, preventing Tat-induced fluid secretion, directly limits a specific mechanism of HIV-1-related diarrhoea. Our data support a 'zinc approach' in adjunct to specific antiretroviral therapy in HIV-1-infected children.

A beneficial reduction of watery diarrhoea-related morbidity induced by zinc supplementation in HIV-1-infected children has recently been demonstrated [1]. In the past few years zinc has emerged as a major therapeutic and preventive strategy against diarrhoea [2–4]. These data now open new perspectives for its use, suggesting that zinc could be a safe, simple and cost-effective tool to reduce morbidity and mortality in HIV-1-infected children [1]. Even though zinc is recommended for the treatment of childhood diarrhoea by WHO/UNICEF [3], several important questions remain to be answered, in particular in the HIV-1-infected child, because the study by Bobat *et al.* [1] did not provide evidence on the mechanisms elicited by zinc to limit diarrhoea in these particular patients.

The aetiology of HIV-1-related severe watery diarrhoea is certainly multifactorial and still largely unknown [5]. We have recently provided evidence for a primary role of Tat (the transactivating peptide produced by HIV-1 and essential for its replication) in the pathogenesis of diarrhoea in AIDS patients. Functioning as an enterotoxin, like *Vibrio cholerae* toxin, Tat stimulates active fluid secretion from the serosal to the luminal side of human enterocytes in the classical Ussing chamber in-vitro model used to investigate transepithelial ion transport [6,7]. A similar mechanism has been reported [8] for *Cryptosporidium parvum*, the most frequent and dangerous opportunistic enteric pathogen in AIDS patients. These findings suggest that HIV-1-infected children are at a high risk of secretory diarrhoea. In addition, we have demonstrated that Tat is able to inhibit sodium ion/glucose symporter activity, a major mechanism to absorb fluid at the intestinal level, further determining the occurrence of diarrhoea [7]. Interestingly, the Tat effects on intestinal ion transport were dose dependent, with a maximal effective dose of 0.1 nmol, which is well within the range of what is generally measured in the sera of patients with HIV-1 infection, suggesting that effective Tat concentrations may well be reached *in vivo* [9,10]. On the other hand, zinc is directly active on transepithelial ion transport at the intestinal level, as direct zinc–enterocyte interaction results in net ion absorption, thereby counteracting active secretion, such as that induced by *V. cholerae* toxin in the same experimental model [11]. To determine whether zinc was effective in inhibiting Tat-induced ion secretion, we incubated human derived Caco-2 intestinal cells with chemical synthesized, high-pressure liquid chromatography 96% pure, HIV-1 Tat, (Tecnogen, Piana di Monteverna, Italy), in the presence or absence of zinc ($ZnSO_4$; Sigma Chemical Co., St Louis, Missouri, USA), using the Ussing chamber experimental model [6]. As shown in Figure 1, the pre-incubation of human enterocytes with

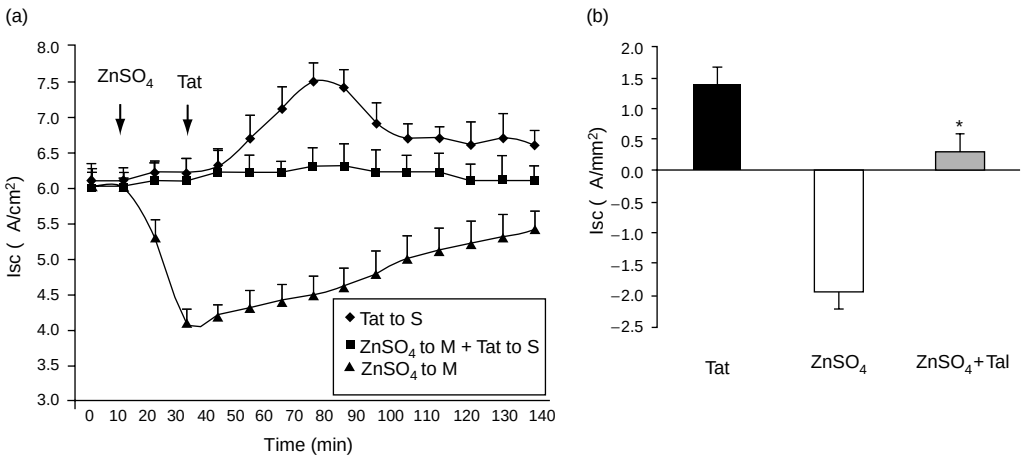

Fig. 1. Zinc inhibitory effect against HIV-1 Tat protein-induced intestinal ion secretion. (a) Time course of the effect of HIV-1 Tat protein and $ZnSO_4$ addition, alone or in combination, on transepithelial ion transport in human enterocyte (Caco-2 cell) monolayer mounted in Ussing chambers (i.e. an established in-vitro model to study ion transport). Tat addition to the enterocytes serosal side (S) induced an increase in the intensity of short circuit current (Isc), indicating the presence of active chloride ion secretion. $ZnSO_4$ determined a pure pro-absorptive effect on transepithelial chloride ion transport (i.e. a decrease in Isc). Pre-incubation for 20 min with $ZnSO_4$ to the mucosal side (M) was able significantly to reduce the secretory response elicited by Tat at intestinal level. The arrows indicate the time of addition either of Tat or $ZnSO_4$. (b) Maximal Isc modifications after Tat and $ZnSO_4$ addition, alone or in combination, to human enterocytes mounted in Ussing chambers. Data are expressed as mean ± SE, and significance was evaluated by the non-parametric, two-tailed Mann–Whitney *U* test. $*P < 0.001$ Tat alone versus $ZnSO_4$ plus Tat. ◆ Tat to S; ■ $ZnSO_4$ to M plus Tat to S; ▲ $ZnSO_4$ to M.

zinc (35 μmol) resulted in the almost total inhibition of Tat-induced ion secretion, as reflected by the intensity of the short circuit current. These results suggest that zinc is able to prevent intestinal fluid secretion induced by Tat, and is able to interact directly with a specific mechanism of HIV-1-related diarrhoea, explaining well the results obtained in South Africa by Bobat and co-workers [1], and supporting the usefulness of the 'zinc approach' in adjunct to specific antiretroviral therapy in HIV-1-infected children.

An emphasis on the costs and economic benefits of an alternative therapy is an important aspect of health services research. The cost savings and the attractive cost-effectiveness, disposability and thermostability of zinc indicates the need to assess further the role of these micronutrients in the prevention and treatment of diarrhoea in AIDS patients.

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