Table 1. Drug resistance mutations revealed in plasma and peripheral blood mononuclear cells.

Source	RT	PR	Source	RT	PR
Plasma, undiluted	**77FI**, 100FIL, 101K, 106IL, 108LV, **118DEV**, **215ST**	**30DEHQ**, 33V, 63S	**PBMC undiluted**	**41LM, 67DN, 118IV, 184MV, 210LV, 215NSTY**	10I, 33LV, 36IM, **46IM, 54IV**, 63FLPS, 77IV, **82CFGV**
Dilution 1 : 1000			**PBMC dilution 1 : 150**		
Regions amplified nos. 7, 18, 23, 25, 27, 29, 31, 32, 36b, 37, 40	None	33V 63S	Regions amplified nos. 45, 46, 49, 53, 58, 62, 68, 73, 74, 75, 77, 79	None	33V 63S
Region amplified no. 22	None	33V 36I 63S	Region amplified no. 14	**41LM, 67DN, 118IV 181HY, 184GMRV, 210LW, 215NSTY**	10IL, 20IKM, 33LV, 36IM, **46IM, 54IV**, 63FLPS, 77IV **82CFGV**
			PBMC dilution 1 : 450		
			Regions amplified nos. 19, 30, 40	None	33V 63S
			Region amplified no. 24	**67N, 184V, 210W, 215Y**	36I
			Region amplified no. 32	**M41L, D67N, V118I**	10I, 20I, 36I, **46I, 54V**, 77I, **82C**

PBMC, Peripheral blood mononuclear cell; PR, protease; RT, reverse transcriptase.
Primary mutations in bold, the code number of each amplified region is reported.

not present in those patients with low levels of DR viruses.

In our patient, the genotype shows partial compromise of the potency of a wide variety of drugs, also commonly used for first-line therapy. The presence of wild type as a prevalent replicating strain allowed us to obtain complete suppression before the resistant strain begins to replicate from the archived compartment.

Finally, a therapy with a high genetic barrier, and sufficiently potent, is necessary in the initial phase of treatment, both in the acute and in the chronic phase of infection, to overcome the possible emergence of archived resistance mutations that may compromise the response to treatment.

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Received: 12 October 2005; accepted: 27 October 2005.

References

1. Barbour JD, Hecht FM, Wrin T, Liegler TJ, Ramstead CA, Busch MP, *et al*. **Persistence of primary drug resistance among recently HIV-1 infected adults.** *AIDS* 2004; **18**:1683–1689.
2. Pao D, Andrady U, Clarke J, Dean G, Drake S, Fisher M, *et al*. **Long term persistence of primary genotypic resistance after HIV-1 seroconversion.** *J Acquir Immune Defic Syndr* 2004; **37**:1570–1573.
3. Hecht FM, Grant RM. **Resistance testing in drug-naive HIV-infected patients: is it time?** *Clin Infect Dis* 2005; **41**:1324–1325.
4. Parisi SG, Zazzi M, Dal Bello F, Manfrin V, Nicolini G, Carolo G, *et al*. **HIV-proviral DNA and plasma-RNA sequencing for detection of drug resistance mutations in antiretroviral naive patients.** In: *Program and Abstracts of the 3rd IAS Conference on HIV Pathogenesis and Treatment*. Rio de Janeiro, 2005 [Poster presentation TuPp0304].
5. Verhofstede C, Noe A, Demecheleer E, De Cabooter N, Van Wanzeele F, Van Der Gucht B, *et al*. **Drug-resistant variants that evolve during nonsuppressive therapy persist in HIV-1-infected peripheral blood mononuclear cells after long-term highly active antiretroviral therapy.** *J Acquir Immune Defic Syndr* 2004; **35**:473–483.

Compartment-specific HIV-1 resting T-cell reservoirs

A proof-of-concept study recently assessed the efficiency of a standard clinical dose of valproic acid in order selectively to induce the expression of HIV-1 latent proviral genome, and potentially to deplete HIV from the blood resting CD4 T-cell reservoir [1]. The efficacy of this purge of the CD4 T-cell reservoir by valproic acid has been hypothesized to be tissue compartment specific [2]. We conducted a study on HIV-1 T-cell reservoirs in the blood and breast milk of nine HIV-1-infected women. Using an exquisitely sensitive HIV-1 antigen enzyme-linked immunospot (ELISPOT) assay combined with the quantitation of HIV-1 DNA by real time polymerase

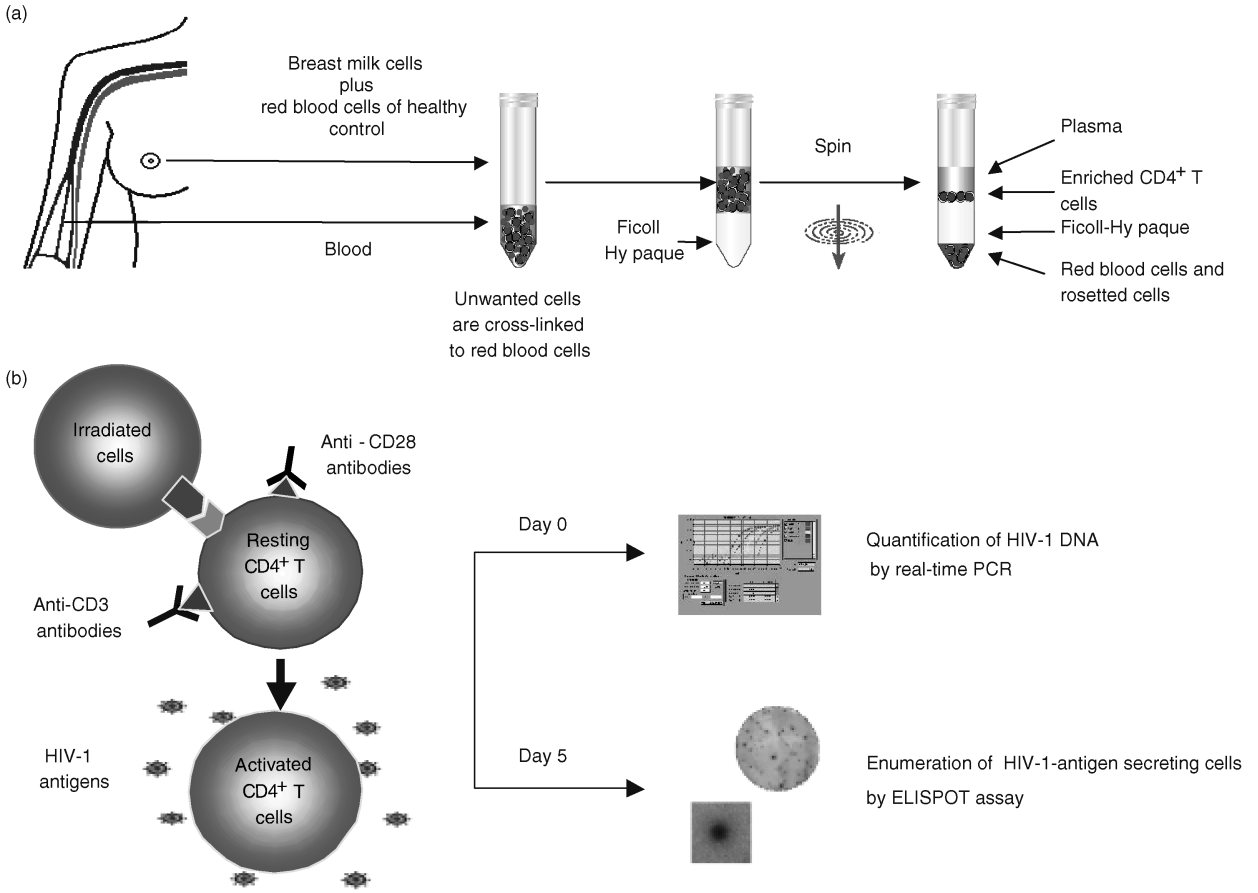

Fig. 1. Characterization of resting T-cell reservoir in blood and breast milk. (a) Isolation of resting CD4 T cells from blood and breast milk samples. (b) Quantification of the HIV-1 DNA and enumeration of HIV-1 antigen-secreting cells after CD4 T-cell polyclonal activation. ELISPOT, Enzyme-linked immunospot; PCR, polymerase chain reaction.

chain reaction (see Fig. 1), we were able to demonstrate a 17 times higher capacity of HIV-1 latently infected CD4 T cells to enter the replicative cycle after ex-vivo activation, in breast milk compared with blood [3]. The study is important not only for understanding the possible mechanisms of HIV-1 transmission by breastfeeding, but also for anticipating the feasibility of an HIV cure by treatments aimed at depleting the HIV-1 proviral reservoirs.

First, our HIV-1 antigen ELISPOT assay is at least as sensitive and considerably less time consuming and labour intensive than the limiting-dilution culture of resting CD4 T cells after ex-vivo activation, as used in the study by Lehrman *et al.* [1]. Major points concerning our approach consist of the combination of: (i) an efficient method of resting CD4 T-cell isolation; (ii) the conservation of CD4 T-cell functions; and (iii) an exquisitely sensitive ELISPOT assay (with the capacity to detect a single HIV-1 antigen-producing cell among 1×10^6 CD4 T lymphocytes) [4]. As it can be applied to other compartments than blood, the HIV-1 antigen ELISPOT assay may become the technique of choice for further studies on the compartmentalization of the HIV-1 resting CD4 T-cell reservoirs.

Second, our study demonstrates a strong compartmentalization of the HIV-1 latently infected CD4 T-cell reservoir at least in the mammary gland, because HIV-1-infected resting CD4 T cells can replicate HIV-1 after ex-vivo activation more extensively in breast milk than in blood.

Compartmentalization of the viral reservoir is not an academic fantasy. For example, a strong genotypic and phenotypic compartmentalization of cell-free as well as cell-associated HIV-1 has been observed in the blood and seminal reservoirs after an intensification and stimulatory HIV-1 eradication protocol [5]. As suggested by Routy [2], the enumeration and assessment of functional capacities of resting CD4 T cells to enter a replicative cycle in peripheral blood may not necessarily reflect what happens in other anatomical or tissue compartments, such as seminal fluid, cervicovaginal secretions, central nervous

system or breast milk. In our ultimate goal of finding a cure for HIV, this compartmentalization should be taken into account in further clinical trials on valproic acid or related compounds aimed at purging the HIV-1 proviral reservoir.

The authors declare that they have no conflict of interest.

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Sponsorship: This work was supported by the Agence Nationale de Recherches sur le SIDA (ANRS), as part of the project ANRS 1271 (contract 2004-010).

Received: 19 January 2006; accepted: 7 February 2006.

References

1. Lehrman G, Hogue IB, Palmer S, Jennings C, Spina CA, Wiegand A, *et al.* **Depletion of latent HIV-1 infection *in vivo*: a proof-of-concept study.** *Lancet* 2005; **366**:549–555.
2. Routy JP. **Valproic acid: a potential role in treating latent HIV infection.** *Lancet* 2005; **366**:523–524.
3. Becquart P, Petitjean G, Al Tabaa Y, Valéa D, Huguet MF, Tuaillon E, *et al.* **Detection of a large T cell reservoir able to actively replicate HIV-1 in breast milk**. *AIDS* 2005; **20**: in press.
4. Fondere JM, Petitjean G, Huguet MF, Sahli SL, Baillat V, Biegum AM, *et al.* **Human immunodeficiency virus type 1 (HIV-1) antigen secretion by latently infected resting CD4+ T lymphocytes from HIV-1-infected individuals.** *J Virol* 2004; **78**:10536–10542.
5. Nunnari G, Leto D, Sullivan J, Xu Y, Mehlman KE, Kulkosky J, Pomerantz RJ. **Seminal reservoirs during an HIV type 1 eradication trial [Short communication].** *AIDS Res Hum Retroviruses* 2005; **21**:768–775.

Spontaneous HIV-1 replication in a B-lymphoblastoid cell line obtained from an HIV-1-positive patient with undetectable plasma viral load

Combined antiretroviral treatment (ART) has improved the survival rate and retarded progression to AIDS in HIV-1-infected patients by reducing active viral replication and allowing immune reconstitution [1]. Nevertheless, the source of re-emerging virus after ART interruption is difficult to establish. Reservoirs of viral persistence include latently infected CD4 T memory cells, tissue macrophages and pockets of continuous low key HIV-1 replication in different tissues [2,3]. Resting peripheral blood B lymphocytes are poor targets for HIV-1 infection under normal conditions, but an activated immunological environment could induce the expression of HIV-1 receptors CD4 and CXCR4 on B lymphocytes, increasing their susceptibility to infection [4,5].

We now report the occurrence of a spontaneous B lymphoblastoid cell line (B-LCL) producing sustained high levels of infectious HIV-1 (20 000 pg/ml p24 accumulated in the supernatant after 20 days) for over 34 months. This B-LCL overgrew a non-stimulated peripheral blood mononuclear cell (PBMC) culture [6] from a haemophilic HIV-1-positive patient receiving continuous zidovudine–lamivudine–indinavir treatment for 4 years preceding the appearance of the HIV-1-positive B-LCL (CDK) with an undetectable HIV-1 plasma viral load (< 50 HIV-1-RNA copies/ml). No HIV-1 was produced by monocytes/macrophages (M/M) or T lymphocytes in PBMC cultures during the 4–8 weeks of culture preceding the spontaneous overgrowth of Epstein–Barr virus (EBV)-positive B lymphoblasts. On previous occasions prolonged non-stimulated culture of PBMC from this patient resulted in the overgrowth of 19 different B-LCL, but in contrast to CDK, HIV-1 replication was not detected or it lasted for a short time at low levels in two out of 19 B-LCL. To date, the patient has had a satisfactory clinical evolution, with few complications and a stable CD4 cell count (240–500 CD4 T lymphocytes/μl). He was originally classified as belonging to the Centers for Disease Control and Prevention C2 group [7] after an episode of interstitial pneumonia of probable *Pneumocystis carinii* origin. Tuberculosis was detected and treated after 6 months of this.

CDK B-LCL was characterized by flow cytometry, and its phenotype corresponded to that of EBV-positive B-LCL: CD19+, CD20+, CD23+, CD30+, CCR5−, less than 1% CD4+, 5% CXCR4+, EBV LMP-1+. The presence of HIV-1 in CDK B-LCL was demonstrated by p24 detection and confirmed by in-situ hybridization (Fig. 1a) and by electron microscopy. The continuous release of HIV-1 was assessed by p24 detection, and the presence of infectious HIV-1 virions in CDK B-LCL supernatants was confirmed by assays of infection using different cell targets: MT-2 cell line for X4 strains, primary non-stimulated cultures for M/M-tropic strains (R5) [8], and EBV-positive B-LCL for atypical non-X4/non-R5 strains. CDK culture supernatants preferentially infected EBV-positive B-LCL when compared with T-cell lines or primary PBMC (Fig. 1b). HIV-1 from CDK supernatants evidenced the non-nucleoside reverse transcriptase inhibitor mutation 103N conferring resistance to nevirapine, delavirdine and efavirenz (generously tested by Dr H. Salomon, CNRS, Facultad de Medicina, Universidad de Buenos Aires, using VircoTYPE HIV-1; Mechelen, Belgium).