



HAL
open science

HIV controllers exhibit potent CD8 T cell capacity to suppress HIV infection ex vivo and peculiar cytotoxic T lymphocyte activation phenotype

Asier Sáez-Ciri3n, Christine Lacabaratz, Olivier Lambotte, Pierre Versmisse, Alejandra Urrutia, Faroudy Boufassa, Franoise Barr3-Sinoussi, Jean-Franois Delfraissy, Martine Sinet, Gianfranco Pancino, et al.

► To cite this version:

Asier Sáez-Ciri3n, Christine Lacabaratz, Olivier Lambotte, Pierre Versmisse, Alejandra Urrutia, et al.. HIV controllers exhibit potent CD8 T cell capacity to suppress HIV infection ex vivo and peculiar cytotoxic T lymphocyte activation phenotype. Proceedings of the National Academy of Sciences of the United States of America, 2007, 104 (16), pp.6776-6781. 10.1073/pnas.0611244104. pasteur-00142658

HAL Id: pasteur-00142658

<https://pasteur.hal.science/pasteur-00142658v1>

Submitted on 23 Apr 2007

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destin3e au d3p3t et 3 la diffusion de documents scientifiques de niveau recherche, publi3s ou non, 3manant des 3tablissements d'enseignement et de recherche franais ou 3trangers, des laboratoires publics ou priv3s.

1 **Classification:** Biological Sciences - Immunology

2

3 **HIV controllers exhibit potent CD8 T cell capacity to**
4 **suppress HIV infection *ex vivo* and peculiar CTL**
5 **activation phenotype**

6

7 Asier S3ez-Ciri3n*[¶], Christine Lacabartz[¶], Olivier Lambotte^{†,‡}, Pierre Versmisse*,
8 Alejandra Urrutia[†], Faroudy Boufassa[§], Franoise Barr3-Sinoussi*, Jean-Franois
9 Delfraissy^{†,‡}, Martine Sinet[†], Gianfranco Pancino*[#] and Alain Venet^{†#}; the ANRS EP36
10 HIV CONTROLLERS study group.

11

12 *Institut Pasteur, Unit3 de R3gulation des Infections R3trovirales, Paris, France; [†]Inserm
13 U802, Facult3 de M3decine Paris XI, Le Kremlin-Bic3tre, France; [‡]Service de M3decine
14 Interne et Maladies Infectieuses, H3pital Bic3tre, Le Kremlin-Bic3tre, France; and
15 [§]Inserm/Ined U569, H3pital Bic3tre, Le Kremlin-Bic3tre, France.

16 [¶]Both first and [#]both last authors contributed equally to this work

17

18 **Correspondence to:** Gianfranco Pancino, Unit3 de R3gulation des Infections
19 R3trovirales, Institut Pasteur, 25 rue du Dr Roux, 75725 Paris Cedex 15, France. Tel:
20 +33 145688738; Fax: +33 145688957; e-mail: gpancino@pasteur.fr; Alain Venet,
21 INSERM U802, Facult3 de M3decine Paris XI, 63, rue Gabriel P3ri, 94276 Le Kremlin
22 Bic3tre Cedex, France. Tel: + 33 149596719; Fax: + 33 149596724; e-mail:
23 alain.venet@u-psud.fr

1 **Manuscript information:** 26 text pages, 1 table and 4 figures, plus 3 supplemental
2 figures. 215 words in abstract. 38839 total characters.

3

4

5 *Abbreviations*

6 CFSE, carboxyfluorescein diacetate succinimidyl ester ; CTL, cytotoxic T lymphocytes
7 ; HIC, HIV controllers ; IFN, interferon ; IL-2, interleukin-2 ; PBMC, peripheral blood
8 mononuclear cells ; PHA, phytohaemagglutinin ; SFC, Spot-Forming Cells.

1 **ABSTRACT**

2 Some rare HIV-1-infected individuals, referred to as HIV controllers, have persistently
3 undetectable plasma viral load in the absence of therapy. This control of HIV-1
4 replication has been associated with a strong, multifunctional specific CD8+ T cell
5 response. However, no direct link between this immune response and the control of
6 viremia has so far been provided.

7

8 We investigated parameters of specific CD8 T cell response and *in vitro* susceptibility
9 to HIV-1 infection in 11 HIV controllers. We found high frequencies of HIV-specific
10 CD8+ T cells. Interestingly, these cells expressed the activation marker HLA-DR but
11 not CD38. This unique phenotype differentiates HIV-specific CD8 T cells from HIV
12 controllers and non-controller subjects and likely reflects a high potential to expand
13 upon exposure to antigen and a capacity to exert effector functions. Accordingly,
14 although their CD4+ T cells were fully susceptible to HIV-1 superinfection, CD8+ T
15 cells from HIV controllers effectively suppressed HIV-1 infection. Remarkably, this
16 potent anti-HIV activity was observed without prior stimulation of CD8+ T cells. It was
17 not mediated by secreted inhibitory factors, was due to the elimination of infected
18 CD4+ T cells and was only observed with autologous CD4+ T cells, indicating an HLA-
19 restricted cytotoxic mechanism. This constitutive antiviral capacity of CD8+ T cells
20 could account for the control of viral replication in HIV controllers.

21

22

23

1 **INTRODUCTION**

2 Most untreated HIV-1-infected individuals have continuous viral replication and
3 ultimately progress to AIDS. However, a rare subpopulation of HIV-infected patients
4 spontaneously control viral replication for long periods in the absence of treatment (1-
5 5). These individuals, referred to here as HIV controllers (HIC), are characterized by
6 undetectable plasma HIV-1 RNA. Some HIC have been found to be infected by
7 replication-incompetent viruses (6). However, a potent immune response to HIV-1 is
8 thought to be pivotal in these patients (3, 7, 8).

9
10 Actually, HIC generally exhibit a strong CD8+ T cell specific response and high
11 frequencies of HIV-specific CD8+ T cells despite very low levels of viral antigens (3, 4,
12 7). Furthermore, (i) HIV-specific CD8+ T cells from HIC are qualitatively different
13 from those of progressors (8, 9); (ii) some HLA-Bw4 haplotypes (e.g. B27, B57) are
14 over-represented in HIC (10, 11), suggesting an important role of class I-restricted
15 CD8+ T cells; and (iii) multiepitopic and *de novo* CD8+ T cell responses are associated
16 with suppression of viremia despite CTL escape mutations (12).

17
18 However, the mechanisms by which CD8+ T cells restrain HIV-1 infection in HIC are
19 still unclear. The remarkable spontaneous viral control in HIC offers a unique model in
20 which to shed some light on efficient *in vivo* mechanisms of CD8+ T cell-mediated
21 HIV-1 control. We characterized the parameters of the CD8+ T cell response in 11 HIC
22 from a previously described group (5), providing evidence that their HIV-specific CD8+
23 T cells possess a unique phenotype. In addition, we demonstrated an extraordinary
24 capacity of their CD8+ T cells *ex vivo* to suppress HIV-1 infection.

25

1 **RESULTS**

2 **Study Population**

3 The characteristics of the 11 HIC are reported in Table 1. HLA alleles B57 and B27,
4 associated with protection from progression to AIDS (10, 11), were overrepresented (7
5 and 4 subjects possessed these alleles, respectively).

6

7 **HIV controllers exhibit large numbers of HIV-specific CD8+ T cells with variable** 8 **maturation profiles**

9 High frequencies of HIV-specific CD8+ T cells (7546 ± 5121 SFCs/ 10^6 PBMCs) with a
10 broad response (7 ± 3 peptides recognized) were observed in most HIC (Fig. 1A).
11 Subjects bearing either the HLA-B57 or -B27 allele had the highest frequencies of HIV-
12 specific CD8+ T cells. These frequencies were not significantly different from those of
13 viremic controls (3719 ± 3455 SFCs/ 10^6 PBMCs) but significantly higher than those of
14 HAART subjects (758 ± 1025 SFCs/ 10^6 PBMCs) (Fig. 1B).

15 The maturation status of HIV-specific CD8+ T cells was analyzed by measuring
16 membrane expression of CD45 RO and the differentiation marker CD27. This
17 combination readily identifies effector cells as being CD27- (CD27-/CD45RO+
18 defining classical effector cells and CD27-/CD45RO- [i.e. CD45RA+] the terminally
19 differentiated CD8+ T cells (13)). Overall, higher percentages of CD27- (effector) HIV-
20 specific CD8+ T cells were observed in HIC than in viremic subjects (Fig. 2A).
21 However, the profiles were extremely heterogeneous within HIC (Fig 2A and 5) and
22 only a minority of cells expressed high levels of perforin ($2.3\% \pm 1.6\%$) whatever their
23 maturation profile.

24

25 **HIV-specific HIC CD8+ T cells possess an unusual activation profile**

1 We examined the activation status of CD8⁺ T cells by evaluating HLA-DR and CD38
2 expression. HIV-specific CD8⁺ T cells showed low expression of CD38 (15% ± 9%,
3 similar to that observed among the total CD8⁺ T cell population: 19% ± 5%) (Fig. 2B).
4 In contrast, HIV-specific CD8⁺ T cells expressed a high level of HLA-DR (52% ±
5 17%), higher than that observed among total CD8⁺ T cells (25% ± 16%, *P* = 0.001)
6 (Fig. 2B). This may suggest an activation of HIV-specific CD8⁺ T cells, which is
7 unexpected in subjects with undetectable plasma RNA levels, and puzzling in the
8 absence of high CD38 expression (Fig. 6A). Actually, HIV-specific CD8⁺ T cells from
9 viremic subjects exhibited high expression of both CD38 and HLA-DR (51% ± 20%
10 and 46 ± 21%, respectively), whereas those from HAART subjects had low expression
11 of these two markers (11% ± 8% and 26 ± 20% for CD38 and HLA-DR, respectively),
12 (Figure 2C and 6A). In addition to activation, expression of HLA-DR has been
13 associated with proliferation (14). HIV-specific CD8⁺ T cells from HIV controllers had
14 a high proliferation capacity (on average, 60% ± 31% of HIV-specific CD8⁺ T cells that
15 proliferated) that was positively correlated to the expression of HLA-DR (*P* Spearman =
16 0.03)(data not shown). This profile may rather reflect the capacity to proliferate than
17 ongoing proliferation *in vivo*, as Ki-67 expression was low on *ex vivo* HIV-specific
18 CD8⁺ T cells (data not shown). Consistent with a high proliferative potential, in
19 proliferation assays all CFSE^{low} specific CD8⁺ T cells were HLA-DR⁺ (Fig. 6B).
20 Finally, we observed a mutually exclusive expression of HLA-DR and the CD57 marker
21 on HIV-specific CD8⁺ T cells (Figure 6C and data not shown).

22

23 ***Ex vivo* unstimulated HIC CD8⁺ T cells control HIV-1 infection**

24 Seeking a direct link between the particular CD8⁺ T cell response in HIC and the
25 control of viremia, we next evaluated the capacity of circulating HIC CD8⁺ T cells to

1 suppress, in the absence of exogenous stimulation, HIV-1 infection in autologous CD4+
2 T cells.

3 Purified CD4+ T cells from HIC supported viral replication at levels similar to those
4 observed in healthy controls (2520 ± 997 and 2729 ± 1248 ng p24/ml at the peak of
5 viral replication in the 11 HIC and 12 healthy controls, respectively) (Fig. 3A). As
6 expected (15), the levels of viral replication in CD4+ T cells from 10 untreated viremic
7 HIV-1-infected individuals were highly heterogeneous (2331 ± 2204 ng p24/ml) (Fig.
8 3A).

9 When cocultures of CD4+ T cells and autologous unstimulated CD8+ T cells were
10 infected with HIV-1, viral replication was undetectable in 9 out of the 10 HIC tested
11 (4.0 ± 1.1 log p24 decrease, CD8:CD4 vs CD4, n=10) and strongly reduced in the
12 remaining subject, B2 (Fig. 3A) who, interestingly, showed also the lowest HIV-
13 specific CD8+ T cell response (see Fig. 1A: 130 SFC/ 10^6 PBMC, 1 peptide recognized).

14 In contrast, unstimulated CD8+ T cells from healthy donors were devoid of antiviral
15 activity (0.1 ± 0.2 log p24 decrease, CD8:CD4 vs CD4, n=12) and unstimulated CD8+
16 T cells from viremic individuals were never able to efficiently control HIV-1
17 superinfection in autologous CD4+ T cells (0.9 ± 0.6 log p24 decrease, CD8:CD4+
18 1:1 ratio vs CD4, n=9) (Fig. 3A). Furthermore, the antiviral activity of CD8+ T cells
19 from viremic individuals was rapidly lost when CD8+ T cells were diluted, whereas
20 HIC CD8+ T cells were still fully effective at a ratio of 0.1 CD8+ T cell to 1 CD4+ T
21 cell (fig. 3B).

22 This remarkable anti-HIV-1 activity of unstimulated HIC CD8+ T cells was observed
23 with a wide range of infectious doses (BaL HIV-1 m.o.i. = $10^{-2.8}$ to $10^{-0.8}$) (data not
24 shown) both with laboratory-adapted HIV strains and primary isolates, regardless of
25 clade and tropism (HIV-1 NL4.3 (X4); 132W (dual tropic), subtype E; DH12 (dual

1 tropic) and BX08 (R5), subtype B), (data not shown). Experiments performed without
2 exogenous IL-2 (that was regularly present in the coculture medium) showed, as
3 expected, a reduced viral replication in infected CD4+ T cells, but the antiviral capacity
4 of HIC unstimulated CD8+ T cells did not depend on the presence of the cytokine (Fig.
5 7).

6

7 **Soluble factors are not responsible for CD8+ T cell-mediated HIV-1 control in HIV** 8 **controllers**

9 In order to clarify the mechanism(s) responsible for the control of HIV-1 infection in the
10 cells from HIC, we assessed the role of soluble factors (16, 17). In HIC, the control of
11 HIV-1 replication was totally lost when unstimulated CD8+ T cells were separated from
12 autologous CD4+ T cells by semi-permeable membranes (Fig. 4A). By contrast, when
13 mitogen-activated CD8+ T cells were separated from autologous CD4+ T cells by semi-
14 permeable membranes, a (modest) reduction in viral replication mediated by soluble
15 factors, was observed in HIC and also frequently in healthy donors (Fig. 4A). Therefore
16 HIC CD8+ T cells, although able to secrete inhibitory soluble factors upon *in vitro*
17 stimulation, do not exert their potent anti-HIV-1 activity *ex vivo* through this
18 mechanism.

19

20 **CD8+ T cells from HIV controllers eliminate the HIV-1 infected CD4+ T cells**

21 To evaluate whether the observed control of HIV-1 infection was achieved through the
22 physical elimination of infected CD4+ T cells by HIC CD8+ T cells, we removed CD8+
23 T cells from superinfected autologous cocultures. The removal of the CD8+ T cells
24 would be expected to rescue HIV-1 replication in the re-purified CD4+ T cells only if

1 HIV-1 control was caused by a CD8-mediated intracellular block, such as the post-
2 integration inhibition induced by the antiviral factor CAF (18).
3 Single-round infections with pseudotyped HIV-1 particles were used to avoid confusing
4 contribution of subsequent viral replication. A marked control of HIV-1 infection was
5 observed at 48h when HIC unstimulated CD8+ T cells were cocultured with autologous
6 CD4+ T cells (Fig. 4B, left). The coculture was then split and CD8+ T cells were
7 depleted from one of the two resulting cocultures. Two days later a comparable strong
8 reduction of HIV-1 infection was observed in both cultures (figure 4B, center).
9 However, when the two cultures were re-challenged with the HIV-1 pseudotype, the
10 CD8-depleted culture was readily superinfected whereas control of infection was still
11 observed in the non depleted coculture (Fig. 4B, right). These results are compatible
12 with a CD8+ T cell-mediated elimination of infected CD4+ T cells. Consistent with this,
13 the addition of HIC CD8+ T cells to autologous CD4+ T cells that had been infected for
14 2 days with GFP reporter HIV-1 particles, provoked a reduction in the number of
15 infected GFP-positive CD4+ T cells (Fig. 4C).

16

17 **Anti-HIV-1 activity of HIC CD8+ T cells requires contact with autologous CD4+ T** 18 **cells**

19 The precedent results strongly supported that control of HIV-1 infection was mediated
20 by a cytotoxic mechanism. To find out if the HIC CD8+ T cell antiviral activity was
21 MHC-restricted, we assessed the capacity of unstimulated HIC CD8+ T cells to control
22 HIV-1 infection in autologous and heterologous CD4+ T cells. The same HIC CD8+ T
23 cells that were able to control HIV-1 *in vitro* when cocultured with autologous CD4+ T
24 cells, were ineffective when cocultured with heterologous CD4+ T cells both from HIC

1 and from healthy controls (Fig. 4D). These results support an MHC-restricted
2 mechanism of suppression.

3

4 **DISCUSSION**

5 Here we provide the first evidence that HIV-specific CD8+ T cells from HIV controllers
6 are characterized by a unique CD38^{low}/HLA-DR^{high} phenotype, which likely reflects a
7 capacity to proliferate upon antigenic stimulation and exert effector functions.
8 Importantly, we also report that circulating HIC CD8+ T cells are able to efficiently
9 control HIV-1 infection *ex vivo*, without further stimulation, suggesting that this
10 antiviral activity is functional *in vivo*. This CD8+ T cell-mediated control is independent
11 of the secretion of antiviral molecules, requires contact with their matching CD4+ T
12 cells, and is caused by the elimination of infected CD4+ T cells, likely due to HIV-
13 specific CD8+ T cells. This previously undescribed spontaneous capacity of CD8+ T
14 cells to clear the virus through the killing of infected cells is particular to HIV
15 controllers.

16

17 In keeping with previous studies in a comparable group of patients (7, 8), we observed
18 that most HIC possessed high frequencies of IFN γ -secreting HIV-specific CD8+ T cells
19 that, overall, did not differ from those seen in chronically viremic patients and were
20 strikingly higher than those observed in HAART-treated non viremic patients (Fig. 1
21 and (7, 8, 19, 20)). These HIV-specific CD8+ T cells had a broad repertoire, particularly
22 in HLA-B27 and/or HLA-B57-positive individuals.

23

24 The high proliferative potential observed in HIC has been previously proposed (8)
25 together with high functionality (9) as hallmarks of a high-quality HIV-specific CD8 T+

1 cell response in HIC. However, no phenotypical differences had been found so far that
2 might distinguish HIC from other patients and be associated with an effective immune
3 response. We evaluated the differentiation status of HIC HIV-specific CD8+ T cells as
4 the skewed maturation of these cells may partly explain the lack of effective control of
5 HIV replication in most HIV-infected patients (21-25). We observed that HIV-specific
6 CD8+ T cells were indeed more differentiated in HIC than in HIV-viremic patients (21,
7 26). Nevertheless, variable profiles were observed within and among the HIC, and,
8 therefore, optimal differentiation of their specific CD8+ T cells does not appear as a
9 distinctive element to explain the effective control of infection.

10
11 The activation phenotype of HIV-specific CD8+ T cells in HIC had not been previously
12 defined. We and others have shown a positive correlation between the level of viral
13 replication and the activation status of HIV-specific CD8+ T cells (26-28). Accordingly,
14 and in contrast to viremic controls, CD38 expression by HIV-specific CD8+ T cells
15 from HIV controllers was very low in this study. Conversely, HLA-DR expression was
16 remarkably high, differently from HAART patients, and much higher than that observed
17 on the total CD8+ T cell population. An increased HLA-DR expression associated with
18 a low CD38 expression had been reported on the global CD8+ T cell population in
19 asymptomatic HIV-infected patients with stable CD4+ T cell counts (29). In HIV
20 controllers we observed that the discordance between the expression of CD38 and HLA-
21 DR was much more pronounced on HIV-specific CD8+ T cells what is puzzling in the
22 context of high and long-lasting viral control. The low expression of CD38 may reflect
23 the lack of general immune activation and the expression of HLA-DR may actually
24 characterize T cells with high proliferative potential. This hypothesis is supported by
25 several data: (i) although we found little evidence of *in vivo* cycling or proliferation, as

1 very few HIV-specific CD8+ T cells expressed Ki67, HIV-specific CD8+ T cells from
2 HIV controllers had potent *in vitro* proliferative capacity upon exposure to antigen; (ii)
3 most HLA-DR^{pos} HIV-specific CD8+ T cells did not express the senescence marker
4 CD57 which lack of expression has been clearly linked to proliferative capacity (30);
5 (iii) finally, we observed a correlation between HLA-DR expression and the percentage
6 of proliferating CFSE^{low} cells among HIV-specific CD8+ T cells.

7

8 Although the control of HIV-1 replication in HIC has been associated with a strong,
9 multifunctional specific CD8 T cell response, the anti-HIV activity of HIC CD8+ T
10 cells had not been thoroughly addressed and a direct link between the immune response
11 and the control of viremia was lacking. First, we show that HIC CD4+ T cells are highly
12 susceptible to HIV-1 *in vitro* infection, discarding, therefore, that an intrinsic resistance
13 of CD4+ T cells to HIV-1 could contribute to HIC status. Second, we found that HIC
14 purified CD8+ T cells were able to efficiently control HIV-1 infection *in vitro* of
15 autologous CD4+ T cells, even if 10 times more diluted than the CD4+ T cells. Our
16 results are particularly relevant since our experiments were conducted with *ex vivo*
17 CD8+ T cells, in the absence of mitogen-activation, showing that CD8+ T cells
18 circulating in the blood of HIC are suitably prepared to control HIV-1 infection. The
19 remarkable spontaneous anti-HIV capacity was a homogeneous feature among CD8+ T
20 cells from HIC, except for patient B2, for whom other factors may contribute to the
21 control of HIV infection. Differences in the CD8 T cell anti-viral activity between HIC
22 and viremic individuals were striking since *none* of the 9 viremic controls tested
23 suppressed the viral replication as opposed to 9 out of 10 HIC tested.

24

1 Although CD8+ T cells in HIV patients have been reported to be able to suppress HIV
2 infection through the secretion of soluble factors (reviewed in (17)) or HLA-class I
3 restricted cytotoxicity (31-33), the relative weight of these mechanisms is controversial.
4 We demonstrate that the ability of HIC CD8+ T cells to control HIV-1 infection *ex vivo*
5 is not linked to the secretion of soluble factors and required contact with infected CD4+
6 T cells. The contact of CD8+ T cells with CD4+ T cells did not induce the production of
7 a restriction factor in the latter but rather the elimination of the infected cells. This
8 antiviral activity was effective on autologous CD4+ T cells only. Altogether, our results
9 point to HLA-restricted cytotoxicity as the mechanism associated to the in vivo control
10 of infection achieved by HIV controllers.

11

12 In conclusion, we provide direct evidence that circulating CD8+ T cells from HIC are
13 spontaneously able to control HIV-1 infection, a striking difference with cells from
14 viremic subjects. Our study strongly suggests a pivotal role of HIV-specific CD8+ T
15 cells in viral control in these individuals. These cells have a unique phenotype which
16 may correspond to activated status *in vivo* but more likely reflects a propensity to
17 expand rapidly upon antigen exposure. This capacity is probably linked to an intact
18 ability to secrete IL-2 and other cytokines (9, 34) and may explain the maintenance of
19 high frequencies of HIV-specific CD8+ T cells in these subjects. The generation of this
20 optimal CD8+ T cell profile is a major research focus. Further studies should provide
21 new insights into the precise mechanisms of HIV control and may serve to a better
22 design of future vaccination or immune-based therapies. Already, both the expression of
23 HLA-DR and CD38 on HIV-specific CD8+ T cells and their capacity to suppress ex
24 vivo HIV-1 might be used as surrogate markers to evaluate the efficiency of induced
25 CTL responses in vaccine trials.

1

2 **MATERIALS AND METHODS**

3 **Study subjects**

4 Eleven patients infected by HIV-1 for > 10 years who had never received antiretroviral
5 treatment and in whom more than 90% of plasma HIV RNA load tests gave values <400
6 copies/ml (HIV controllers) were studied here: 9 have been described elsewhere (5), and
7 2 were newly recruited. All are infected with HIV-1 group M, clade B. HLA-typing was
8 performed by genotype analysis (I. Theodorou, INSERM U543, Paris, France).

9 Among HIV-infected patients recruited and monitored at CHU Kremlin-Bicêtre, France,
10 42 who had an exhaustive evaluation of their HIV-specific CD8+ T cells served as
11 controls : 24 were untreated viremic patients [HIV plasma RNA > 7,500 copies/mL]
12 and 18 were HAART-treated, virologically controlled patients [HIV plasma RNA <200
13 copies/mL].

14 Twelve healthy controls were blood donors from the Etablissement Franais du Sang,
15 Paris, France.

16 All subjects gave their written informed consent.

17

18 **Isolation of primary cells**

19 CD4+ and CD8+ cells were purified (>99%) from freshly isolated peripheral blood
20 mononuclear cells (PBMC) by positive selection with antibody-coated magnetic beads
21 (Miltenyi Biotech, France). CD4+ cells were stimulated for 3 days with
22 phytohaemagglutinin (PHA) at 1 µg/ml in the presence of interleukin-2 (IL-2) (Chiron,
23 France) at 100 IU/ml. The culture medium was RPMI 1640 containing 10% FCS and
24 penicillin/streptomycin (100 U/ml). CD8+ T cells were kept in culture without mitogens
25 or cytokines.

1

2 **Viruses and productive infection *in vitro***

3 Results shown were obtained performing infections *in vitro* with HIV-1 BaL (R5) at
4 m.o.i. $10^{-2.8}$. CD4⁺ T cells (10^5) were infected in triplicate in 96-well plates with a
5 spinoculation protocol (35). For coculture, 10^5 CD4⁺ T cells were mixed with 10^5
6 CD8⁺ T cells (CD8/CD4 ratio 1:1) or with 10^4 CD8⁺ T cells (CD8/CD4 ratio 0.1:1) at
7 the moment of infection. After infection the cells were washed and cultured for 14 days.
8 HIV-1 replication was monitored every 3-4 days in supernatants by p24 ELISA
9 (Beckman-Coulter, France). Unless otherwise indicated, infectivity assays were carried
10 out in the presence of 100 IU/ml IL2.

11

12 **Single-round infections**

13 BaL and VSV-G pseudotyped HIV-1 particles were produced by co-transfecting
14 (SuperFect, Qiagen) 293T cells with the proviral pNL-Luc-E-R⁺ (36) or pNL-GFP-E-
15 R⁺ (37) and BaL-Env or VSV-G expression vectors. CD4⁺ T cells (10^5) were infected
16 in triplicate with HIV-1 pseudotypes, as above. Luciferase activity was assessed in cell
17 lysates (Luciferase Reporter 1000 Assay System, Promega France) in a Veritas
18 microplate luminometer (Turner BioSystems, CA, USA) as described (38).

19

20 **Peptides**

21 We used a set of 124 peptides corresponding to known optimal CTL epitopes (NIH HIV
22 Molecular Immunology Database: <http://www.hiv.lanl.gov/content/immunology/index.html>.
23 The peptides were synthesized by Neosystem (Strasbourg, France). They were used at a
24 final concentration of 2 μ g/mL.

25

1 **Elispot assay**

2 Interferon (IFN)- γ secretion by HIV-specific CD8⁺ T cells was quantified with an
3 ELISPOT assay using appropriate stimuli (HLA-defined optimal peptides derived from
4 the HIV-1 Env, Gag, Pol and Nef proteins or controls) (20). IFN- γ spot-forming cells
5 (SFCs) were counted with a KS-ELISPOT system (Carl Zeiss Vision) and expressed as
6 SFCs/10⁶ PBMC after subtracting the background. Wells were considered positive if
7 they contained at least 50 SFCs/10⁶ PBMC and exhibited at least two times the
8 background level.

9

10 **Antibodies**

11 The following antibodies were used: CD8-ECD or -PC5 (clone B9.11), CD3-PC5
12 (UCHT1), CD45RO-ECD (UCHL1), HLA-DR-ECD (Immu-357), CD38-FITC (T16),
13 and CD57-FITC (NC1) all from Beckman Coulter (San Diego, CA); and CD27-FITC
14 (M-T271), perforin-FITC (dG9) and Ki67-FITC (B56) from BD Biosciences (San Jose,
15 CA).

16

17 **Tetramer staining and phenotyping**

18 HIV-specific CD8⁺ T cells were detected with the following PE-conjugated tetramers:
19 HLA-A*0201-SLYNTVATL (HIV gag 77-85), A*0201-ILKEPVHGV (HIV pol 476-
20 484), A*0301-RLRPGGKKK (HIV gag 20-28), A*0301-QVPLRPMTYK (HIV nef 73-
21 82), and B*2705-KRWIILGLNK (HIV gag 263-272) from Proimmune Ltd (Oxford,
22 UK) and B*5701-KAFSPEVIPMF (HIV gag 162-172) from Beckman Coulter
23 Immunomics. PBMC were incubated with tetramers (1 μ g/mL) for 30 min and then
24 with relevant antibodies for 15 min. Cells were washed in Cell Wash (BD Biosciences)
25 plus 1% BSA, incubated for 10 min with FACS lysing solution (BD Biosciences) and

1 washed. For intracellular staining, cells were incubated for 10 min with FACS
2 permeabilizing solution (BD Biosciences) before adding antibodies for 30 min. Cells
3 were fixed in 1% paraformaldehyde for flow cytometry with a Beckman Coulter Epics
4 XL cytometer and RXP software (Beckman Coulter).

5

6 **Proliferation assay**

7 The proliferative capacity of HIV-specific CD8⁺ T cells was evaluated by flow
8 cytometry. PBMC were stained with 0.35 μ M carboxyfluorescein diacetate
9 succinimidyl ester (CFSE, Molecular Probes, The Netherlands) for 10 min at 37°C, and
10 then stimulated for 5 days with 2 μ g/mL peptide or medium alone. After labelling with
11 tetramer, anti-CD8, anti-CD3 and/or anti-HLA-DR antibodies, PBMC were fixed in 1%
12 paraformaldehyde.

13

14 **Statistical analyses**

15 All values throughout the text are expressed as means \pm standard deviation. P values
16 were calculated with the Mann-Whitney U test. Correlations were identified by simple
17 linear regression analysis and Spearman's rank correlation test.

18

19

20 **ACKNOWLEDGEMENTS**

21 The authors thank Laurence Meyer from the SEROCO/HEMOCO cohort, Ioannis
22 Theodorou and Christine Rouzioux for contributing data, and the physicians who care
23 for the patients. We especially thank the subjects who participated in this study for their
24 cooperation.

1 This work was supported by the French National Agency for Research on AIDS and
2 Viral Hepatitis (ANRS). ASC and CL were the recipients of postdoctoral fellowships
3 from Sidaction and ANRS respectively.

4

5 Authors declare that they have no competing interest.

6

1 **REFERENCES**

- 2 1. Cao, Y., Qin, L., Zhang, L., Safrit, J. & Ho, D. D. (1995) *N Engl J Med* **332**,
3 201-8.
- 4 2. de Quiros, J. C., Shupert, W. L., McNeil, A. C., Gea-Banacloche, J. C.,
5 Flanigan, M., Savage, A., Martino, L., Weiskopf, E. E., Imamichi, H., Zhang, Y.
6 M., et al. (2000) *J Virol* **74**, 2023-8.
- 7 3. Harrer, T., Harrer, E., Kalams, S. A., Elbeik, T., Staprans, S. I., Feinberg, M. B.,
8 Cao, Y., Ho, D. D., Yilma, T., Caliendo, A. M., et al. (1996) *AIDS Res Hum*
9 *Retroviruses* **12**, 585-92.
- 10 4. Kloosterboer, N., Groeneveld, P. H., Jansen, C. A., van der Vorst, T. J., Koning,
11 F., Winkel, C. N., Duits, A. J., Miedema, F., van Baarle, D., van Rij, R. P., et al.
12 (2005) *Virology* **339**, 70-80.
- 13 5. Lambotte, O., Boufassa, F., Madec, Y., Nguyen, A., Goujard, C., Meyer, L.,
14 Rouzioux, C., Venet, A. & Delfraissy, J. F. (2005) *Clin Infect Dis* **41**, 1053-6.
- 15 6. Wang, B., Dyer, W. B., Zaunders, J. J., Mikhail, M., Sullivan, J. S., Williams,
16 L., Haddad, D. N., Harris, G., Holt, J. A., Cooper, D. A., et al. (2002) *Virology*
17 **304**, 246-64.
- 18 7. Gea-Banacloche, J. C., Migueles, S. A., Martino, L., Shupert, W. L., McNeil, A.
19 C., Sabbaghian, M. S., Ehler, L., Prussin, C., Stevens, R., Lambert, L., et al.
20 (2000) *J Immunol* **165**, 1082-92.
- 21 8. Migueles, S. A., Laborico, A. C., Shupert, W. L., Sabbaghian, M. S., Rabin, R.,
22 Hallahan, C. W., Van Baarle, D., Kostense, S., Miedema, F., McLaughlin, M., et
23 al. (2002) *Nat Immunol* **3**, 1061-8.

- 1 9. Betts, M. R., Nason, M. C., West, S. M., De Rosa, S. C., Migueles, S. A.,
2 Abraham, J., Lederman, M. M., Benito, J. M., Goepfert, P. A., Connors, M., et
3 al. (2006) *Blood* **107**, 4781-9.
- 4 10. Flores-Villanueva, P. O., Yunis, E. J., Delgado, J. C., Vittinghoff, E.,
5 Buchbinder, S., Leung, J. Y., Ugliandolo, A. M., Clavijo, O. P., Rosenberg, E. S.,
6 Kalams, S. A., et al. (2001) *Proc Natl Acad Sci U S A* **98**, 5140-5.
- 7 11. Migueles, S. A., Sabbaghian, M. S., Shupert, W. L., Bettinotti, M. P., Marincola,
8 F. M., Martino, L., Hallahan, C. W., Selig, S. M., Schwartz, D., Sullivan, J., et
9 al. (2000) *Proc Natl Acad Sci U S A* **97**, 2709-14.
- 10 12. Bailey, J. R., Williams, T. M., Siliciano, R. F. & Blankson, J. N. (2006) *J Exp*
11 *Med* **203**, 1357-69.
- 12 13. Hamann, D., Kostense, S., Wolthers, K. C., Otto, S. A., Baars, P. A., Miedema,
13 F. & van Lier, R. A. (1999) *Int Immunol* **11**, 1027-33.
- 14 14. Speiser, D. E., Migliaccio, M., Pittet, M. J., Valmori, D., Lienard, D., Lejeune,
15 F., Reichenbach, P., Guillaume, P., Luscher, I., Cerottini, J. C., et al. (2001) *Eur*
16 *J Immunol* **31**, 459-66.
- 17 15. Nethe, M., Berkhout, B. & van der Kuyl, A. C. (2005) *Retrovirology* **2**, 52.
- 18 16. Cocchi, F., DeVico, A. L., Garzino-Demo, A., Arya, S. K., Gallo, R. C. &
19 Lusso, P. (1995) *Science* **270**, 1811-5.
- 20 17. Levy, J. A. (2003) *Trends Immunol* **24**, 628-32.
- 21 18. Mackewicz, C. E., Blackbourn, D. J. & Levy, J. A. (1995) *Proc Natl Acad Sci U*
22 *S A* **92**, 2308-12.
- 23 19. Dalod, M., Dupuis, M., Deschemin, J. C., Sicard, D., Salmon, D., Delfraissy, J.
24 F., Venet, A., Sinet, M. & Guillet, J. G. (1999) *J Virol* **73**, 7108-16.

- 1 20. Lacabartz-Porret, C., Urrutia, A., Doisne, J. M., Goujard, C., Deveau, C.,
2 Dalod, M., Meyer, L., Rouzioux, C., Delfraissy, J. F., Venet, A., et al. (2003) *J*
3 *Infect Dis* **187**, 748-57.
- 4 21. Appay, V., Dunbar, P. R., Callan, M., Klenerman, P., Gillespie, G. M., Papagno,
5 L., Ogg, G. S., King, A., Lechner, F., Spina, C. A., et al. (2002) *Nat Med* **8**, 379-
6 85.
- 7 22. Appay, V., Nixon, D. F., Donahoe, S. M., Gillespie, G. M., Dong, T., King, A.,
8 Ogg, G. S., Spiegel, H. M., Conlon, C., Spina, C. A., et al. (2000) *J Exp Med*
9 **192**, 63-75.
- 10 23. Champagne, P., Ogg, G. S., King, A. S., Knabenhans, C., Ellefsen, K., Nobile,
11 M., Appay, V., Rizzardi, G. P., Fleury, S., Lipp, M., et al. (2001) *Nature* **410**,
12 106-111.
- 13 24. Hess, C., Altfeld, M., Thomas, S. Y., Addo, M. M., Rosenberg, E. S., Allen, T.
14 M., Draenert, R., Eldrige, R. L., van Lunzen, J., Stellbrink, H. J., et al. (2004)
15 *Lancet* **363**, 863-6.
- 16 25. van Baarle, D., Kostense, S., Hovenkamp, E., Ogg, G., Nanlohy, N., Callan, M.
17 F., Dukers, N. H., McMichael, A. J., van Oers, M. H. & Miedema, F. (2002)
18 *AIDS* **16**, 2001-11.
- 19 26. Doisne, J. M., Urrutia, A., Lacabartz-Porret, C., Goujard, C., Meyer, L., Chaix,
20 M. L., Sinet, M. & Venet, A. (2004) *J Immunol* **173**, 2410-8.
- 21 27. Kestens, L., Vanham, G., Gigase, P., Young, G., Hannel, I., Vanlangendonck,
22 F., Hulstaert, F. & Bach, B. A. (1992) *Aids* **6**, 793-7.
- 23 28. Papagno, L., Spina, C. A., Marchant, A., Salio, M., Rufer, N., Little, S., Dong,
24 T., Chesney, G., Waters, A., Easterbrook, P., et al. (2004) *PLoS Biol* **2**, 173-185.

- 1 29. Giorgi, J. V., Ho, H. N., Hirji, K., Chou, C. C., Hultin, L. E., O'Rourke, S., Park,
2 L., Margolick, J. B., Ferbas, J. & Phair, J. P. (1994) *J Infect Dis* **170**, 775-81.
- 3 30. Brenchley, J. M., Karandikar, N. J., Betts, M. R., Ambrozak, D. R., Hill, B. J.,
4 Crotty, L. E., Casazza, J. P., Kuruppu, J., Migueles, S. A., Connors, M., et al.
5 (2003) *Blood* **101**, 2711-20.
- 6 31. Walker, B. D., Chakrabarti, S., Moss, B., Paradis, T. J., Flynn, T., Durno, A. G.,
7 Blumberg, R. S., Kaplan, J. C., Hirsch, M. S. & Schooley, R. T. (1987) *Nature*
8 **328**, 345-8.
- 9 32. Walker, B. D. & Plata, F. (1990) *Aids* **4**, 177-84.
- 10 33. Yang, O. O., Kalams, S. A., Rosenzweig, M., Trocha, A., Jones, N., Koziel, M.,
11 Walker, B. D. & Johnson, R. P. (1996) *J Virol* **70**, 5799-806.
- 12 34. Lichtenfeld, M., Kaufmann, D. E., Yu, X. G., Mui, S. K., Addo, M. M.,
13 Johnston, M. N., Cohen, D., Robbins, G. K., Pae, E., Alter, G., et al. (2004) *J*
14 *Exp Med* **200**, 701-12.
- 15 35. O'Doherty, U., Swiggard, W. J. & Malim, M. H. (2000) *J Virol* **74**, 10074-80.
- 16 36. Connor, R. I., Chen, B. K., Choe, S. & Landau, N. R. (1995) *Virology* **206**, 935-
17 44.
- 18 37. Amara, A., Vidy, A., Boulla, G., Mollier, K., Garcia-Perez, J., Alcami, J.,
19 Blanpain, C., Parmentier, M., Virelizier, J. L., Charneau, P., et al. (2003) *J Virol*
20 **77**, 2550-8.
- 21 38. Sáez-Ciri3n, A., Nicola, M. A., Pancino, G. & Shorte, S. L. (2006) *Biotechnol J*
22 **1**, 682-689.
- 23
24
25

1 **FIGURE LEGENDS**

2 **Figure 1: Frequencies of HIV-specific IFN γ -secreting CD8⁺ T cells in HIV**
3 **controllers, viremic and HAART subjects.**

4 (A) For all subjects (except A5) an average of 41 ± 9 individual peptides were tested,
5 depending on the results of HLA typing. Each bar corresponds to the sum of SFC/10⁶
6 PBMC obtained with peptides described to be restricted by HLA-B57 (orange), HLA-
7 B27 (blue) or other HLA antigens (yellow). * For subject A5, whose HLA-typing was
8 lacking at the time of the study, 12 pools of optimal peptides were used instead of
9 individual peptides. A5 bar corresponds to the sum of SFC/10⁶ PBMC obtained with
10 these pools. (B) Comparison of SFCs (mean \pm SD) in PBMC from HIC, viremic and
11 HAART subjects. Statistical differences in SFC numbers between groups are indicated
12 on the top of the panel.

13

14 **Figure 2: Differentiation and activation status of HIV-specific CD8⁺ T cells from**
15 **HIC and comparison with viremic and HAART subjects.**

16 (A) Individual HIV-specific CD8⁺ CD27⁻ T cell frequencies on CD8⁺ tetramer^{pos} cells
17 (left) in 9 HIC (each symbol represents one individual, 1 to 3 specificities tested per
18 subject) and viremic subjects. Median percentages are represented by a black line. For
19 HIC, results obtained with B*2705-gag 263-272 and B*5701-gag 162-172 tetramers are
20 represented in blue and orange respectively. (B) Frequencies of CD38 (blue) and HLA-
21 DR (red) expression on total CD8⁺ T cells (horizontal bars) and HIV-specific CD8⁺ T
22 cells (circles) in 9 HIC. (C) Comparison of CD38 and HLA-DR expression (median,
23 25th-75th and 10th-90th percentiles) on HIV-specific CD8⁺ T cells in HIC, viremic and
24 HAART groups. Statistical differences between groups are indicated on the top of the
25 panels.

1

2 **Figure 3: Control of HIV-1 infection by *ex vivo* HIC CD8+ T cells**

3 HIV-1 *in vitro* infection assays were done with cells from HIC, uninfected donors
4 (control), or HIV viremic individuals [median plasma viral load was 25,350 RNA
5 copies/ml (range 7,800-321,000)] (viremic). (A) PHA-activated CD4+ T cells were
6 infected, in the absence (gray) or in the presence (white) of autologous unstimulated
7 CD8+ T cells (1:1 ratio), with the replicative HIV-1 BaL. Circles represent the average
8 (n=3 independent infections) peak p24 values for each studied individual. Horizontal
9 lines indicate median values for each group. Statistical differences in CD8+ T cell-
10 mediated inhibition between groups are indicated on the top of the panel. (B) PHA-
11 activated CD4+ T cells (gray bars) and cocultures of autologous unstimulated CD8+ T
12 cells and PHA-activated CD4+ T cells (1:1 ratio, white bars; 0.1:1 ratio, patterned bars)
13 from 5 HIC, 5 uninfected blood donors (control), and 9 HIV viremic individuals
14 (viremic) were infected with HIV-1 BaL. One representative experiment (HIC A9) is
15 shown. Bars represent peak levels of p24 in supernatants (mean \pm SD, n=3). Values
16 below the dashed lines were at background level.

17

18 **Figure 4: Mechanism of HIC CD8+ T cells mediated control of HIV-1 infection.**

19 (A) CD4+ T cells from HIV controllers and from uninfected donors were infected with
20 replicative HIV-1 BaL. CD4+ T cells cultured alone are shown as a reference (gray
21 bars). Autologous unstimulated CD8+ T cells were added directly to the CD4+ T cell
22 culture (blue open bars) or to Transwell inserts that were placed in the CD4+ T cell-
23 containing well (blue patterned bars). Autologous PHA-stimulated CD8+ T cells were
24 added to Transwell (orange patterned bars). The ratio of CD8+ to CD4+ T cells was 1:1.
25 One representative experiment with HIV controller A1 and one uninfected control is

1 shown. Results are peak p24 levels. (B) CD4⁺ T cells from HIV controller A4 were
2 infected with a single-round HIV-1 BaL pseudotype bearing the luciferase gene alone
3 (gray) or in coculture (1:1 ratio) with unstimulated autologous CD8⁺ T cells (white).
4 Results (mean \pm SD, n=3) are the percentage of infection, relative to luciferase activity
5 detected in lysates of infected CD4⁺ T cells, 48 hours after infection (left). At this time
6 point, CD8⁺ T cells from half the coculture were depleted and viral replication was
7 analysed two days later (center). Both the CD8:CD4 coculture (open bars) and the re-
8 purified CD4⁺ T cells (patterned bars) were then re-challenged with the HIV-1 BaL
9 pseudotype and luciferase activity was measured 48 hours later (right). (C) CD4⁺ T
10 cells from HIV controllers were infected with a HIV-1 VSV-G pseudotype bearing the
11 GFP reporter gene. 48 hours later autologous unstimulated CD8⁺ T cells were added to
12 half of the CD4⁺ T cells (white, 1:1 ratio). At the indicated time points an aliquot of
13 each cell suspension was labelled with anti-CD4 antibodies and the quantity of double
14 positive CD4⁺GFP⁺ was assessed by flow cytometry. One representative experiment
15 with HIC A7 is shown. (D) Unstimulated CD8⁺ T cells from HIC (A1, A3 and B5)
16 (circles) and uninfected blood donors (squares) were cocultured (1:1 ratio) with
17 autologous or heterologous HIC CD4⁺ T cells (A1:A3, A3:A1 and B5:A7, CD8:CD4 T
18 cell cultures) or uninfected controls CD4⁺ T cells and infected with replicative HIV-1
19 BaL. Results (average of 3 independent infections) are peak p24 levels.

20
21