HAL
open science

A Subset of Extreme Human Immunodeficiency Virus (HIV) Controllers Is Characterized by a Small HIV Blood Reservoir and a Weak T-Cell Activation Level

Etienne Canouï, Camille Lécuroux, Véronique Avettand-Fenoel, Marine Gousset, Christine Rouzioux, Asier Saez-Cirion, Laurence Meyer, Faroudy Boufassa, Olivier Lambotte, Nicolas Noël, et al.

▶ To cite this version:

Etienne Canouï, Camille Lécuroux, Véronique Avettand-Fenoel, Marine Gousset, Christine Rouzioux, et al.. A Subset of Extreme Human Immunodeficiency Virus (HIV) Controllers Is Characterized by a Small HIV Blood Reservoir and a Weak T-Cell Activation Level. Open Forum Infectious Diseases, 2017, 4 (2), pp.ofx064. 10.1093/ofid/ofx064 . pasteur-02549908

HAL Id: pasteur-02549908

https://pasteur.hal.science/pasteur-02549908v1

Submitted on 21 Apr 2020

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 destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Distributed under a Creative Commons Attribution - NonCommercial 4.0 International License

A subset of extreme HIV controllers is characterized by a small HIV blood reservoir and a weak T cell activation level

Etienne Canouï[1,2,3,4], Camille Lécuroux[2,3,4], Véronique Avettand-Fenoël[5] , Marine Gousset[5], Christine Rouzioux[5], Asier Saez-Cirion[6], Laurence Meyer[4,7,8], Faroudy Boufassa[4,7], Olivier Lambotte[1,2,3,4*] , Nicolas Noël[1,2,3,4,*§] and the ANRS CO21 CODEX study group

[1] Assistance Publique – Hôpitaux de Paris, Service de Médecine Interne et Immunologie Clinique, Groupe Hospitalier Universitaire Paris Sud, Hôpital Bicêtre, Le Kremlin-Bicêtre, France

[2] INSERM UMR 1184, Immunologie des Maladies Virales et Autoimmunes (IMVA), Université Paris Sud, Le Kremlin Bicêtre, France

[3] CEA, DSV/iMETI, Division of Immuno-Virology, IDMIT, France

[4] Université Paris Sud, Le Kremlin Bicêtre, France

[5] Université Paris Descartes, Sorbonne Paris Cité, Faculté de Médecine, EA 7327 Paris, France APHP, Laboratoire de Virologie, Hôpital Necker-Enfants Malades, Paris, France.

[6] Institut Pasteur, Unité HIV inflammation et persistance, Paris, France

[7] INSERM CESP U1018, Centre de recherche en Epidémiologie et Santé des Populations, Le Kremlin-Bicêtre, France

[8] Assistance Publique – Hôpitaux de Paris, Service d'Epidémiologie et de Santé Publique, Hôpitaux Universitaires Paris Sud, Hôpital Bicêtre, le Kremlin-Bicêtre, France

* These authors contributed equally

© The Author 2017. Published by Oxford University Press on behalf of Infectious Diseases Society of America.
This is an Open Access article distributed under the terms of the Creative Commons Attribution-NonCommercial-NoDerivs licence (http://creativecommons.org/licenses/by-nc-nd/4.0/), which permits non-commercial reproduction and distribution of the work, in any medium, provided the original work is not altered or transformed in any way, and that the work is properly cited. For commercial re-use, please contact journals.permissions@oup.com

Accepted Manuscript

[§] Corresponding author

Dr. Nicolas Noel, Service de Médecine Interne et Immunologie Clinique, CHU Bicêtre, 78 rue du Général Leclerc, F-94275 Le Kremlin-Bicêtre cedex, France. Phone: +33 145 212 783, Fax: +33 145 212 733. nicolas.noel@aphp.fr

Accepted Manuscript

ABSTRACT

Background: HIV controllers (HICs) form a heterogeneous group of patients with regard to formal definitions, immunologic characteristics and changes over time in viral load.

Patients and methods: HICs with undetectable viral load (uHICs, ie for whom a viral load had never been detected with routine assays, n=52) were compared with 178 HICs with blips during the follow-up (bHICs). Clinical characteristics, ultrasensitive HIV-RNA and HIV-DNA loads, HIV1-Western blot (WB) profiles and immune parameters were analyzed.

Results: Relative to bHICs, uHICs had significantly lower ultrasensitive plasma HIV-RNA loads ($p<0.0001$) and HIV-DNA levels in PBMC ($p=0.0004$), higher CD4+ T cell count ($p=0.04$) at enrolment, and lower T cell activation levels. Between diagnosis and inclusion in the cohort, the CD4+ T cell count had not changed in uHICs but had significantly decreased in bHICs. 21% of the uHICs lacked specific anti-HIV IgG antibodies; and these individuals also had very low levels of HIV-DNA. Half the uHICs had a protective HLA allele (-B57/58/B27), a weak CD8+ T cell response and very small HIV-DNA reservoir.

Conclusion: We suggest that an interesting HIC phenotype combines protective HLA alleles, low level of HIV blood reservoirs and reduced immune activation. Prospective studies aimed at evaluating the benefit of cART in HIV controllers might take into account the identification of uHICs and bHICs.

Keywords: HIV controllers; HLA; ultrasensitive plasma HIV-RNA load; HIV-DNA in PBMC; immune activation

Accepted Manuscript

BACKGROUND

A small proportion of HIV-infected patients ("HIV controllers", HICs) present with a spontaneous control of HIV replication [1]. Since the initial description of HICs [2,3], a variety of definitions have been suggested. These definitions differ in terms of the length of follow-up, the threshold for the HIV-RNA load (VL), and the number of VL measurements required [4].

The mechanisms that underlie spontaneous viral control in HICs have not been fully characterized. Viral control is a complex phenomenon that combines several host and/or viral factors. Most HICs harbor replication-competent viruses [5,6]. The genetic background is probably involved in HIC status, since the human leukocyte antigen (HLA)-B57/B58 and -B27 protective alleles are over-represented among HICs [7,8]. HICs exhibit strong antiviral immune responses mediated by polyfunctional CD4+ and CD8+ T cells [9,10]. Some HICs exhibit a peculiar activation phenotype, with low CD38 and high HLA-DR expression [9]; this might generate HIV-specific CD8+ T cells that are capable of rapid viral suppression [11]. We have shown that the HICs' HIV-suppressing capacity is related to the magnitude of HIV-specific CD8+ T cell responses [12]. However, some HICs with weak CD8+ T cell HIV-suppressive responses are still able to sustainably control the viral infection [12]. Based on their CD8+ T cells' ability to suppress HIV infection *ex vivo*, HICs can be classified into strong and weak responders (SRs and WRs); the level of response is closely related to the size of the viral reservoir and the probability of viral reactivation [12,13]. Indeed, HIV controllers have lower total HIV-DNA levels than patients on combined antiretroviral therapy (cART), and the size of blood reservoirs levels seems to be associated with the risk of viral progression [14,15]. Lastly, it was recently shown that HICs had significantly higher levels of antibody-

Accepted Manuscript

dependent cellular cytotoxicity (ADCC) than viremic subjects – suggesting that ADCC has a role in HLA-B57-negative HICs [16].

Although HICs have a number of common features, they are heterogeneous with regard to some immunologic characteristics and changes over time in the VL levels. Indeed, some HICs display a fall over time in their CD4+ T cell count or lose the ability to control HIV [14,17,18]. These changes may be subtended by excessive immune activation [14,18–20]. Recent studies of HICs with different VL cutoff levels have highlighted the presence of various virologic and immunologic profiles [4,21,22]. Here, our objective was to describe HICs with the most extreme phenotype of viral control measuring residual replication and blood reservoir levels in association with the levels of immune activation and HIV antibodies responses.

Accepted Manuscript

PATIENTS AND METHODS

The study population comprised 230 HICs from the ongoing ANRS CO21 CODEX cohort enrolled from 2009 to 2012 in different French sites. HICs were defined as antiretroviral-therapy (ART)-naïve, HIV-1-infected patients with at least 5 consecutive VL measurements below 400 copies/mL over a period of at least 5 years [20]. We recorded retrospectively all previous CD4+ and CD8+ T cell counts, plasma HIV-RNA (VL) loads and demographic, epidemiologic and other clinical data. The subjects had HLA genotyping at inclusion in the cohort using a complement-mediated lymphocytotoxicity test (InGen Biosciences, France). Patients were followed up every 6 to 12 months. For CD4 and CD8 T cell activation parameters, data from uninfected donors (HD, n=29) were analyzed on blood samples available for the Etablissement Français du Sang. For inflammatory biomarkers, data from patients on ART (defined by > 2 years of HIV VL < 40 copies/mL on therapy) were extracted from our previous study [20].

The study protocol was approved by the regional investigational review board (*Comité de Protection des Personnes Ile-de-France VII*, Paris, France; approval reference: 05–22) and performed in compliance with the tenets of the Declaration of Helsinki.

HIV-RNA loads were measured on sites with different real-time PCR-based assays; depending on the date of enrolment in the cohort and the assay routinely used on each site, the VL detection limit varied from 500 to 10 copies/mL. We defined two different HIC phenotypes as a function of the routinely measured VLs. The first group comprised HICs in whom the VL had never exceeded the detection threshold of the routine assay until last follow-up (December 31st, 2013). We referred these patients with stringent undetectable VL as uHICs (n=52). Detectable VLs were allowed if detected within the 12 months after the diagnosis of

Accepted Manuscript

HIV infection (presumably corresponding to the primary infection period), if they were followed by a spontaneous control to undetectable levels. This was observed in three out of the 52 uHICs. The remaining HICs (meeting the ANRS CODEX criteria but with one or more VL blips above the assay's threshold) were referred to as HICs with "blips" (bHICs, n=178).

Whole blood samples and peripheral blood mononuclear cells (PBMCs, isolated on a Ficoll-Hypaque gradient) were cryopreserved at enrolment. Total cell associated HIV-DNA levels in PBMC were quantified using the real time PCR GENERIC HIV DNA cell assay (Biocentric, Bandol, France) [23]. The threshold of the ultra-sensitive technique was below 10 copies per 10^6 PBMCs . To better quantify low level viral replication, plasma HIV-RNA loads were assayed using an ultrasensitive, real-time PCR technique (GENERIC HIV Charge virale, Biocentric, Bandol, France) [23] with a threshold ranging from 1 to 13 copies/mL, depending on the available plasma volume.

CD4+ cell counts were determined by flow cytometry, using standard procedures.

Levels of cytokines and chemokines (IP10, sCD14, sCD163 and ultrasensitive CRP), surface expression of T-lymphocyte activation markers (HLA-DR and CD38), levels of interferon-gamma (IFN-γ), and ADCC activity were determined according to published protocols [12,16,20,24]. The method used to measure CD8+ T cells' ability to suppress HIV-1 infection of autologous CD4+ T cells *ex vivo* has been previously described [25]. Briefly, this index corresponded to the log drop in p24 production when superinfected CD4+ T cells were cultured in the presence of autologous CD8+ T cells. If a 2 log or greater decrease was observed (relative to baseline), HICs were classified as strong responders (SRs). If the decrease was less than 2 log units, they were classified as weak responders (WRs) [12]. Serum levels of IgG directed against HIV-1 antigens were analyzed by HIV-1 Western blot

Accepted Manuscript

(BIORAD, France). Semi-quantification was performed by scanning bands using the Azurespot Software.

Continuous variables were expressed as the median [interquartile range (IQR)], and categorical variables were expressed as the frequency (n) and percentage (%). Intergroup differences in continuous variables were assessed using a Mann-Whitney U test, Kruskall-Wallis test or Student's t test, depending on the data distribution. For intergroup comparisons, a Dunn's correction was performed after Kruskal-Wallis analysis to take into account multiple comparisons when applicable. Categorical variables were compared using a chi-squared test or Fischer's exact test, as appropriate. A linear mixed-effects model was used to estimate changes over time in the CD4+ T cell count. T_0 was defined as the date of inclusion in the cohort, and we back-modelled the changes in CD4+ T cell counts until the first available measurement. The model took account of the fact that patients had undergone repeated CD4+ T cell counts. The threshold for statistical significance was set to $p<0.05$. Data were stored and analyzed using PRISM software (version 5, GraphPad Software, La Jolla, CA) and Stata software (version 14.0, 2015; Stata Corp., College Station, TX).

Accepted Manuscript

RESULTS

By assessing the 230 HICs included in the ANRS CO21 CODEX cohort at the time of the study, we identified 52 uHICs and 178 bHICs. There were no significant differences between the uHIC and bHIC groups in terms of age, gender, the year of HIV diagnosis (**Table 1**), HCV/HBV coinfections and the route of infection (data not shown). The median year of enrolment of uHICs and bHICs in the CODEX cohort was 2010, and they had been followed up for a median [IQR] of respectively 18 [12-24] and 16 [10-24] years after HIV diagnosis (p=0.15). The proportion of patients with protective HLA alleles was higher in the uHIC group than the bHIC group; this was true for B57/B58/B27 alleles overall (uHICs: 70%; bHICs: 55%; p=0.05) and the B57 allele in particular (uHICs: 50%; bHICs: 33%; p=0.03).

uHICs have lower residual replication and total cell associated HIV-DNA loads than bHICs

In addition to the routinely determined HIV-RNA loads, uHICs and bHICs differed significantly in terms of the median [IQR] usHIV-RNA load (<4 copies/mL [<2-<4] vs. 21 [7-84] copies/mL, respectively; p<0.0001) and the proportion of patients with an undetectable ultrasensitive HIV-RNA at enrolment (80% vs. 24%, respectively; p<0.0001). Moreover, uHICs had significantly lower levels of HIV blood reservoirs than the bHICs, as measured by total cell associated HIV-DNA load (<10 copies per 10^6 PBMCs [<10-11] vs. 21 [<10-52], respectively; p=0.0004) and the proportion of patients with an undetectable HIV-DNA level at enrolment (60% vs. 26%, respectively; p=0.0006).

Immunologic characteristics of uHICs and bHICs

As shown in **Table 1**, uHICs had a higher CD4+ T cell count at enrolment than bHICs. At that time, the median percentage of CD4 in circulating lymphocytes was 43 [36-49] % in uHICs

Accepted Manuscript

and 37 [30-45] % in bHICs (non significant difference). In order to analyze the changes over time in the CD4+ T cell count (from the first available measurement to enrollment in the cohort), we estimated the slope of the decrease in the CD4+ T cell counts (**Figure 1**). In the bHIC group, the slope was -5.16 CD4/µL/year; this value differed significantly from 0 (p=0.001). In contrast, the slope in the uHICs group did not differ significantly from 0 (-3.08 CD4/µL/year; p=0.28). Moreover, the percentage of CD4 T cells remained above 40% all throughout the history of uHICs and below 40% in bHICs using a linear mixed-effects model (**Supplemental Figure 1**).

We compared the frequencies of activated HLA-DR+/CD38+ CD4+ and CD8+ T cells in uHICs and bHICs during their longitudinal follow-up. As shown in **Figure 2 A-B**, uHICs had lower proportion of activated HLA-DR+ CD38+ CD8+ T cell than bHICs at enrolment in the cohort and then 12 and 24 months thereafter. There was no intergroup difference in CD4+ T cell activation. When comparing these parameters to uninfected donors (non-parametric Kruskal-Wallis test followed by Dunn's corrections for multiple comparisons), both uHICs had higher CD4+ and CD8+ T cell activation (p=0.046 and p=0.005, respectively) as well as bHICs (p=0.0009 and p<0.0001, respectively) (**Supplemental Figure 2**), as already suggested by our group and others [11,18]. We then analyzed the correlation between immune activation and ultrasensitive HIV-RNA or -DNA VL. As shown in the **Figure 2 C-D**, the immune activation was significantly correlated with ultrasensitive RNA load for all HICs (Spearman's r = 0.41, p<0.0001 for CD4+ T cell activation; and r = 0.46, p<0.0001 for CD8+ T cell activation). These correlations were preserved for both uHICs (r = 0.54, p=0.0028 and r = 0.56, p=0.0017 for CD4+ and CD8+ T cell activation, respectively) and bHICs (r = 0.36, p=0.0016 and r = 0.31, p=0.0064 for CD4+ and CD8+ T cell activation, respectively).

Accepted Manuscript

We also analyzed the levels of sCD14, IP10 and IL6 between HD, uHICs, bHICs and patients on cART. Data were extracted from our previous studies [20] and HIV controllers were reclassified into uHICs and bHICs according to the current definition. Data were available for 13 uHICs (12 for sCD14) and 56 bHICs, 40 HD and 30 patients on cART. Using Kruskall-Wallis non-parametric analyses for intergroup comparison following by Dunn's correction to take into account the multiple comparisons, we observed that sCD14 levels were significantly higher in bHICs and in cART than in HD (p=0.0001 and p<0.0001, respectively), and IL6 was higher in cART than in uHICs or bHICs (p=0.002 for both). As already published, IP10 levels were higher in uHICs, bHICs and cART than in HDs (p=0.002, p<0.0001 and p<0.0001, respectively) (**Supplemental Figure 2, C-E**). Taken together, these results underline the fact that if present, immune activation in HICs is correlated with levels of ultrasensitive HIV-RNA levels and that uHICs have lower CD8+ T cell activation than bHICs. Moreover, the analysis of inflammatory biomarkers shows that uHICs and bHICs have similar or reduced inflammation than patients on cART, depending on the biomarker considered.

We observed a trend towards lower ADCC activity in uHICs than in bHICs (median [IQR]: 2829 units [586-24570] vs. 15390 [2858-34200], respectively; p=0.07). There was no statistically significant intergroup difference in specific anti-HIV CD8+ T cell responses (measured in IFN-γ ELISpot assays), although a trend was observed for reduced ELISpot responses in uHICs with median values of 530 [16-3533] and 1493 [232-3690] spot-forming cells per 10^6 PBMCs in the uHIC and bHIC groups, respectively (p=0.10).

When considering the CD8+ T cells' ability to control viral replication *in vitro*, no differences were observed between uHICs and bHICs at enrolment (median decrease in p24 production : 0,65 [0,19-2,39] vs. 1,01 [0,35-2,79], p=0,21) and the proportions of WRs and SRs were also similar in both groups (**Table 1**). Among patients with a protective HLA allele (*i.e.* -B57/58

Accepted Manuscript

and/or -B27), the proportion of WRs was higher in the uHIC group than in the bHIC group (52% vs. 33%, respectively; *p*=0.02). All of the WR uHICs carrying a protective HLA allele had no detectable viral replication with an ultrasensitive VL level below the threshold. Moreover, 70% of these patients had HIV-DNA level below the threshold; the median [IQR] value of < 10 [<10-11] copies per 10^6 PBMCs was much lower than in other groups of HICs (*p*=0.0004; **Supplemental Figure 3**).

HICs lacking HIV-specific antibodies have a low level of HIV-DNA

To further investigate the uHIC group, we used semi-quantitative HIV-1 WB to detect IgG antibodies against HIV proteins. Plasma samples collected at enrolment were available for 47 uHICs. As shown in **Figure 3**; we found that 10 of the 47 uHICs (21%) lacked at least one HIV-specific antibody by semi-quantitative analysis and were described as having weak IgG responses. Seven of these 10 uHICs lacked either anti-p68 or anti-p34 antibodies. Overall, these 10 patients had very low HIV-DNA levels (median [IQR]: < 10 copies per 10^6 PBMCs [<9-10], vs. <10 [<10-15] copies per 10^6 PBMCs for uHICs who were seropositive for all the tested anti-HIV antibodies; *p*=0.09).

Most importantly, 83% of these patients had both undetectable HIV-DNA and us HIV-RNA, compared with 40% of uHICs with full anti-HIV IgG responses (**Figure 3**).

In conclusion, we identify a subgroup of HIV controller patients with sustained viral control characterized by low-to-undetectable blood reservoirs and HIV replication based on ultrasensitive techniques. These patients did not experience any significant CD4+ T cell decline on the long term and had low T cell activation and anti-HIV IgG responses.

Accepted Manuscript

DISCUSSION

Although HICs share certain features, they form a heterogeneous group with regard to virologic and immunologic characteristics [4,19,21]. It has been shown that the magnitude of VLs, thresholds and blips are predictive of viral progression in HICs [14,26]. Here, we performed an extensive analysis of HICs with the most stringent viral control phenotype in one of the largest published cohorts, and over a long follow-up period (more than 16 years in median). The present study is the first to have focused on HICs with undetectable VL throughout their follow-up.

We showed that in addition to routinely determined HIV loads, uHICs and bHICs differed in terms of ultrasensitive RNA VL and cellular HIV-DNA levels. We and others have suggested that in some HICs for whom VLs cannot be detected using commercial assays, the use of ultrasensitive HIV-RNA assays can reveal low-level of viral replication [19,27]. The magnitude of low replication level was associated with the likelihood of disease progression [19,27]. Our results are also consistent with reports showing that the level of HIV-DNA in PBMCs is associated with disease progression [15,23,28]. Here, we found that the cellular HIV-DNA level in PBMC was lower in uHICs than in bHICs at the time of inclusion in the cohort (*i.e.,* a median of 16 to 18 years after HIV diagnosis). In the present study, we were only able to explore the HIV reservoirs in blood. It is known that infected cells may also reside in the lymph nodes, gut-associated lymphoid tissue, adipose tissue and/or central nervous system of HIV-infected patients [29–31]. These reservoirs may contribute to the persistence of low-level HIV replication and disease progression in some HICs.

Accepted Manuscript

HIV reservoirs are established very early at the time of primary infection and then slowly diminish during the chronic infection stage in treated patients [15]. In HICs, it is possible that innate mechanisms (such as reduced susceptibility to infection), and/or an optimal peptide-presentation via protective HLAs to CD8+ T cells limit the size of HIV reservoirs and slow viral replication in the first months or years post-infection [11,13,32,33]. We found that half of the uHICs had a protective HLA allele associated with weak CD8+ T cell responses – suggesting that they were experiencing little antigenic stimulation at the time of the analyses. Indeed, these uHICs had the lowest ultrasensitive HIV-RNA loads and the lowest HIV-DNA levels (**Supplemental Figure 3**). Although the role of cell susceptibility to infection and viral replicative capacity could not be investigated in the present study, we, and others, previously showed that HICs are infected with replication-competent viruses [5,6]. In these patients, long-term, sustained, spontaneous viral control (with no viral replication detected in blood) could have reduced levels of T cell activation and allowed the shrinkage of the CD8+ T cell response to a small pool of quiescent HIV-specific memory CD8+ T cells. We recently showed that the long-term spontaneous control of HIV infection in HICs might be related to (i) inefficient reactivation of viruses in a limited number of infected cells and (ii) the HIV-specific CD8+ T cells' ability to activate rapidly and thus limit viral reactivation in this context [13]. In the present study, we found that in 21% of the uHICs lacked at least one of tested anti-HIV antibodies (according to WBs) at the time of enrolment in the cohort (*i.e.* a median of 18 years after diagnosis of the HIV infection) further supporting the lack of recent antigenic stimulation in these patients [34].

A major question remains: are HICs and HIV-infected patients on ART exposed to the same levels of potentially harmful, chronic inflammation? Patients on ART with high CD4+ T cell

Accepted Manuscript

counts and long-term undetectable plasma VLs seem to be less exposed than bHICs to chronic inflammation and its clinical consequences [35]. Although the true frequency of non-AIDS-related diseases in HICs is subject to debate, these comorbidities undoubtedly increase following exposure to blips and the subsequent immune activation [36]. Dominguez-Molina et al. recently described an elevated risk of non-AIDS defining events (notably a cardiovascular risk) during loss-of-control periods in HICs [37]. The persistent, low-grade inflammation observed in some HICs [18,38] might be related not only to the persistence of extremely low tissue levels of viral replication [27,39] but also to other parameters - such as CMV reactivation, microbial translocation due to non-healed epithelial gut lesions having developed early in the course of primary infection, and defects in Th17/Treg subsets - that were not explored in the present study. Levels of monocyte/macrophage activation should be investigated in more detail. In fact, uHICs exhibited similar levels of soluble CD14 (a marker of monocyte activation in the course of HIV infection) than uninfected donors, whereas these levels were higher in bHICs and patients on cART [20]. It is now essential to evaluate the clinical consequences of chronic monocyte inflammation in HICs.

This raises the question of the need for cART in HIV controllers. It has recently been suggested by Bansal et al. that HICs with over 40% of CD4+ T cell count [40] had reduced T cell activation and that high CD4 percentage could be included in the determination of HICs that may have limited benefit from ART. Our results further redound on this sense. CD4% T cells from uHICs remained above 40% in median and CD4+ T cell count slope did not differ significantly from zero throughout all their clinical follow-up. In contrast bHICs had % CD4+ T cells below 40% and significantly declined, albeit slowly, overtime. uHICs had lower T cell activation than bHICs, and T cell activation was correlated with ultrasensitive HIV-RNA,

Accepted Manuscript

which was undetectable in 80% of uHICs. Lastly, inflammatory biomarkers were similar or lower in uHICs and bHICs than in patients on cART. cART could help to reduce the inflammation related to detectable residual replication in bHICs, but the indication of treating the uHICs remains largely questionable.

This study suggests that HICs combining protective HLA alleles, sustained undetectable plasma HIV-RNA loads, low HIV-DNA levels and reduced immune activation present an interesting phenotype of durable control in the absence of therapy. Prospective studies aimed at evaluating the benefit of cART in HIV controllers might take into account the identification of uHICs and bHICs.

NOTES

ACKOWLEDGMENTS

We wish to thank patients and clinicians who participate in the study; we also thank Samia Hendou and Azeb Tadesse for data monitoring, Laurent Tran for data management, and Dr Christine Bourgeois (INSERM U1184) for helpful discussions.

DECLARATION OF INTEREST

All the authors declare that they have no conflict of interest

FUNDING SOURCES

The ANRS Codex cohort is funded by ANRS.

Accepted Manuscript

REFERENCES

1. Okulicz JF, Lambotte O. Epidemiology and clinical characteristics of elite controllers. Curr Opin HIV AIDS. **2011**; 6(3):163–168.

2. Lambotte O, Boufassa F, Madec Y, et al. HIV controllers: a homogeneous group of HIV-1-infected patients with spontaneous control of viral replication. Clin Infect Dis Off Publ Infect Dis Soc Am. **2005**; 41(7):1053–1056.

3. Walker BD. Elite control of HIV Infection: implications for vaccines and treatment. Top HIV Med Publ Int AIDS Soc USA. **2007**; 15(4):134–136.

4. Olson AD, Meyer L, Prins M, et al. An evaluation of HIV elite controller definitions within a large seroconverter cohort collaboration. PloS One. **2014**; 9(1):e86719.

5. Lamine A, Caumont-Sarcos A, Chaix M-L, et al. Replication-competent HIV strains infect HIV controllers despite undetectable viremia (ANRS EP36 study). AIDS Lond Engl. **2007**; 21(8):1043–1045.

6. Blankson JN, Bailey JR, Thayil S, et al. Isolation and characterization of replication-competent human immunodeficiency virus type 1 from a subset of elite suppressors. J Virol. **2007**; 81(5):2508–2518.

7. Sáez-Cirión A, Pancino G, Sinet M, Venet A, Lambotte O, ANRS EP36 HIV CONTROLLERS study group. HIV controllers: how do they tame the virus? Trends Immunol. **2007**; 28(12):532–540.

8. Miura T, Brockman MA, Schneidewind A, et al. HLA-B57/B*5801 human immunodeficiency virus type 1 elite controllers select for rare gag variants associated with reduced viral replication capacity and strong cytotoxic T-lymphocyte [corrected] recognition. J Virol. **2009**; 83(6):2743–2755.

9. Sáez-Cirión A, Lacabaratz C, Lambotte O, et al. HIV controllers exhibit potent CD8 T cell capacity to suppress HIV infection ex vivo and peculiar cytotoxic T lymphocyte activation phenotype. Proc Natl Acad Sci U S A. **2007**; 104(16):6776–6781.

10. Potter SJ, Lacabaratz C, Lambotte O, et al. Preserved central memory and activated effector memory CD4+ T-cell subsets in human immunodeficiency virus controllers: an ANRS EP36 study. J Virol. **2007**; 81(24):13904–13915.

11. Hua S, Lécuroux C, Sáez-Cirión A, et al. Potential role for HIV-specific CD38-/HLA-DR+ CD8+ T cells in viral suppression and cytotoxicity in HIV controllers. PloS One. **2014**; 9(7):e101920.

12. Sáez-Cirión A, Sinet M, Shin SY, et al. Heterogeneity in HIV Suppression by CD8 T Cells from HIV Controllers: Association with Gag-Specific CD8 T Cell Responses. J Immunol. **2009**;

Accepted Manuscript

182(12):7828–7837.

13. Noel N, Peña R, David A, et al. Long-term Spontaneous control of HIV-1 relates to low frequency of infected cells and inefficient viral reactivation. J Virol. **2016**; .

14. Noel N, Lerolle, Nathalie, Lécuroux C, et al. Immunologic and virologic progression in HIV controllers: the role of viral "blips" and immune activation in the ANRS CO21 CODEX study. PloS One Process Publ.

15. Avettand-Fènoël V, Hocqueloux L, Ghosn J, et al. Total HIV-1 DNA, a Marker of Viral Reservoir Dynamics with Clinical Implications. Clin Microbiol Rev. **2016**; 29(4):859–880.

16. Lambotte O, Pollara J, Boufassa F, et al. High antibody-dependent cellular cytotoxicity responses are correlated with strong CD8 T cell viral suppressive activity but not with B57 status in HIV-1 elite controllers. PloS One. **2013**; 8(9):e74855.

17. Okulicz JF, Marconi VC, Landrum ML, et al. Clinical outcomes of elite controllers, viremic controllers, and long-term nonprogressors in the US Department of Defense HIV natural history study. J Infect Dis. **2009**; 200(11):1714–1723.

18. Hunt PW, Brenchley J, Sinclair E, et al. Relationship between T cell activation and CD4+ T cell count in HIV-seropositive individuals with undetectable plasma HIV RNA levels in the absence of therapy. J Infect Dis. **2008**; 197(1):126–133.

19. Boufassa F, Saez-Cirion A, Lechenadec J, et al. CD4 dynamics over a 15 year-period among HIV controllers enrolled in the ANRS French observatory. PloS One. **2011**; 6(4):e18726.

20. Noel N, Boufassa F, Lécuroux C, et al. Elevated IP10 levels are associated with immune activation and low $CD4^+$ T-cell counts in HIV controller patients. AIDS Lond Engl. **2014**; 28(4):467–476.

21. Côrtes FH, Passaes CPB, Bello G, et al. HIV controllers with different viral load cutoff levels have distinct virologic and immunologic profiles. J Acquir Immune Defic Syndr 1999. **2015**; 68(4):377–385.

22. Groves KC, Bibby DF, Clark DA, et al. Disease Progression in HIV-1-Infected Viremic Controllers. J Acquir Immune Defic Syndr 1999. **2012**; 61(4):407–416.

23. Avettand-Fènoël V, Chaix M-L, Blanche S, et al. LTR real-time PCR for HIV-1 DNA quantitation in blood cells for early diagnosis in infants born to seropositive mothers treated in HAART area (ANRS CO 01). J Med Virol. **2009**; 81(2):217–223.

24. Pollara J, Hart L, Brewer F, et al. High-throughput quantitative analysis of HIV-1 and SIV-specific ADCC-mediating antibody responses. Cytom Part J Int Soc Anal Cytol. **2011**; 79(8):603–612.

Accepted Manuscript

25. Sáez-Cirión A, Shin SY, Versmisse P, Barré-Sinoussi F, Pancino G. Ex vivo T cell-based HIV suppression assay to evaluate HIV-specific CD8+ T-cell responses. Nat Protoc. **2010**; 5(6):1033–1041.

26. Madec Y, Boufassa F, Porter K, et al. Natural history of HIV-control since seroconversion. AIDS Lond Engl. **2013**; 27(15):2451–2460.

27. Hatano H, Delwart EL, Norris PJ, et al. Evidence for persistent low-level viremia in individuals who control human immunodeficiency virus in the absence of antiretroviral therapy. J Virol. **2009**; 83(1):329–335.

28. Williams JP, Hurst J, Stöhr W, et al. HIV-1 DNA predicts disease progression and post-treatment virological control. eLife. **2014**; 3:e03821.

29. Yukl SA, Sinclair E, Somsouk M, et al. A comparison of methods for measuring rectal HIV levels suggests that HIV DNA resides in cells other than CD4+ T cells, including myeloid cells. AIDS Lond Engl. **2014**; 28(3):439–442.

30. Hatano H, Somsouk M, Sinclair E, et al. Comparison of HIV DNA and RNA in gut-associated lymphoid tissue of HIV-infected controllers and noncontrollers. AIDS Lond Engl. **2013**; 27(14):2255–2260.

31. Damouche A, Lazure T, Avettand-Fènoël V, et al. Adipose Tissue Is a Neglected Viral Reservoir and an Inflammatory Site during Chronic HIV and SIV Infection. PLoS Pathog. **2015**; 11(9):e1005153.

32. Sáez-Cirión A, Hamimi C, Bergamaschi A, et al. Restriction of HIV-1 replication in macrophages and CD4+ T cells from HIV controllers. Blood. **2011**; 118(4):955–964.

33. Lécuroux C, Sáez-Cirión A, Girault I, et al. Both HLA-B*57 and plasma HIV RNA levels contribute to the HIV-specific CD8+ T cell response in HIV controllers. J Virol. **2014**; 88(1):176–187.

34. Burbelo PD, Bayat A, Rhodes CS, et al. HIV antibody characterization as a method to quantify reservoir size during curative interventions. J Infect Dis. **2014**; 209(10):1613–1617.

35. Rasmussen LD, May MT, Kronborg G, et al. Time trends for risk of severe age-related diseases in individuals with and without HIV infection in Denmark: a nationwide population-based cohort study. Lancet HIV. **2015**; 2(7):e288-298.

36. Crowell TA, Gebo KA, Blankson JN, et al. Hospitalization Rates and Reasons Among HIV Elite Controllers and Persons With Medically Controlled HIV Infection. J Infect Dis. **2015**; 211(11):1692–1702.

37. Dominguez-Molina B, Leon A, Rodriguez C, et al. Analysis of Non-AIDS-Defining Events in HIV Controllers. Clin Infect Dis Off Publ Infect Dis Soc Am. **2016**; 62(10):1304–1309.

Accepted Manuscript

38. Hsue PY, Hunt PW, Schnell A, et al. Role of viral replication, antiretroviral therapy, and immunodeficiency in HIV-associated atherosclerosis. AIDS Lond Engl. **2009**; 23(9):1059–1067.

39. Pereyra F, Palmer S, Miura T, et al. Persistent low-level viremia in HIV-1 elite controllers and relationship to immunologic parameters. J Infect Dis. **2009**; 200(6):984–990.

40. Bansal A, Sterrett S, Erdmann N, et al. Normal T-cell activation in elite controllers with preserved CD4+ T-cell counts. AIDS Lond Engl. **2015**; 29(17):2245–2254.

Accepted Manuscript

TABLES

Table 1. Characteristics of the study population at enrolment in the CODEX cohort.

	uHICs (n=52)	bHICs (n=178)	*p*
Male, n (%)	18 (35)	88 (49)	0.06
Age at HIV diagnosis (years)	29 [25-35]	31 [26-37]	0.29
Year of HIV diagnosis	1996 [1989-2002]	1998 [1990-2004]	0.21
Age at enrolment in the CODEX cohort (years)	45 [40-52]	46 [39-51]	0.83
Year of enrolment	2010 [2009-2011]	2010 [2009-2012]	0.37
Duration of follow-up (years) since HIV diagnosis	18 [12-24]	16 [10-24]	0.15
Protective HLA alleles			
HLA-B57, n (%)	25/50 (50)	58/175 (33)	**0.03**
HLA-B27/57/58, n (%)	35/50 (70)	90/165 (55)	**0.05**
HCV co-infection, n (%)	19 (31.7)	34 (21.0)	0.10
HBV co-infection, n (%)	0	1 (0.6)	1
CD4+ T cell count (cells /µL) at enrolment	790 [638-1038]	711 [520-920]	**0.04**
Ultrasensitive HIV-RNA (copies/mL), n=168	<4 [<2-<4]	21 [7-84]	**<0.0001**
% undetectable ultrasensitive HIV-RNA VL, n=168	33/41 (80)	30/127 (24)	**<0.0001**
HIV-DNA (copies per 10^6 PBMCs), n=136	<10 [<10-11]	21 [<10-52]	**0.0004**
% undetectable HIV-DNA	18/30 (60)	28/106 (26)	**0.0006**
Weak responders (%)	31/44 (70)	105/156 (67)	0.69
WR + protective HLA alleles (%)	23/44 (52)	51/156 (33)	**0.02**

Data are presented as median [IQR] or n (%), respectively. uHICs= "VL undetectable" HICs; bHICs= "with blips" HICs; HCV= Hepatitis C virus; Hepatitis B virus; HCV and HBV status were determined by anti-hepatitis C antibody and hepatitis B surface antigen; WR= Weak responder; VL= viral load

Accepted Manuscript

FIGURE LEGENDS

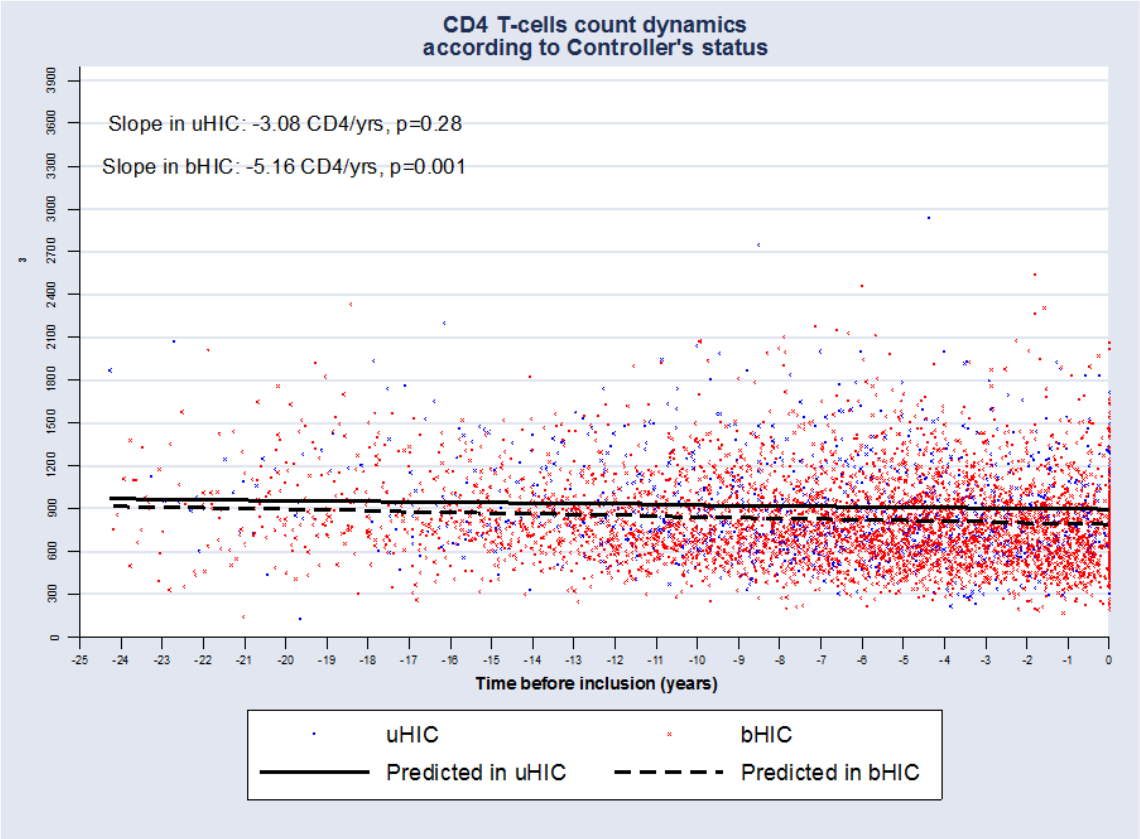
Figure 1: Slopes of CD4+ T cell counts in a linear mixed-effects model, as a function of uHIC or bHIC status.

Figure 2: Immunologic characteristics of the study population, as a function of uHIC or bHIC status. A. and B. Frequencies of HLA-DR+ CD38+ activated CD8+ T cells and CD4+ T cells, respectively, at enrollment in the cohort (n=34 uHICs and 116 bHICs for CD4+ T cells and n=34 and 117 for CD8+ T cells) and then at month (M)12 (n=28 uHICs and 98 bHICs for CD4+ T cells and n=28 and 98 for CD8+ T cells), M24 (n=30 uHICs and 83 bHICs for CD4+ T cells and n=30 and 84 for CD8+ T cells) and M36 (n=27 uHICs and 59 bHICs for CD4+ T cells and n=27 and 59 for CD8+ T cells). *: p=0.05, **: p<0.01. The area of immune activation in uHICs (between X-axis and the full-line) in the time is depicted in the gray zone, whereas the area of immune activation in bHICs is comprised between the X-axis and the dotted line. C and D. Correlations between HLA-DR+ CD38+ CD4+ (C) and CD8+ (D) T cells and ultrasensitive HIV RNA load (Spearman).

Figure 3: A. Heat map representation of serum HIV IgG status (WB analysis testing reactivity for gp160, gp110/120, p68/66, p55, p52/51, gp41, p40, p34/31, p24/25, p18/17) at enrolment for uHICs (n=47). B. Repartition of proportion of undetectable HIV DNA and ultrasensitive HIV RNA, among uHICs with weak or full serum HIV IgG responses. As defined in the text, patients with at least one absent band on semi-quantitative analysis of the western-blot were defined as having weak IgG responses.

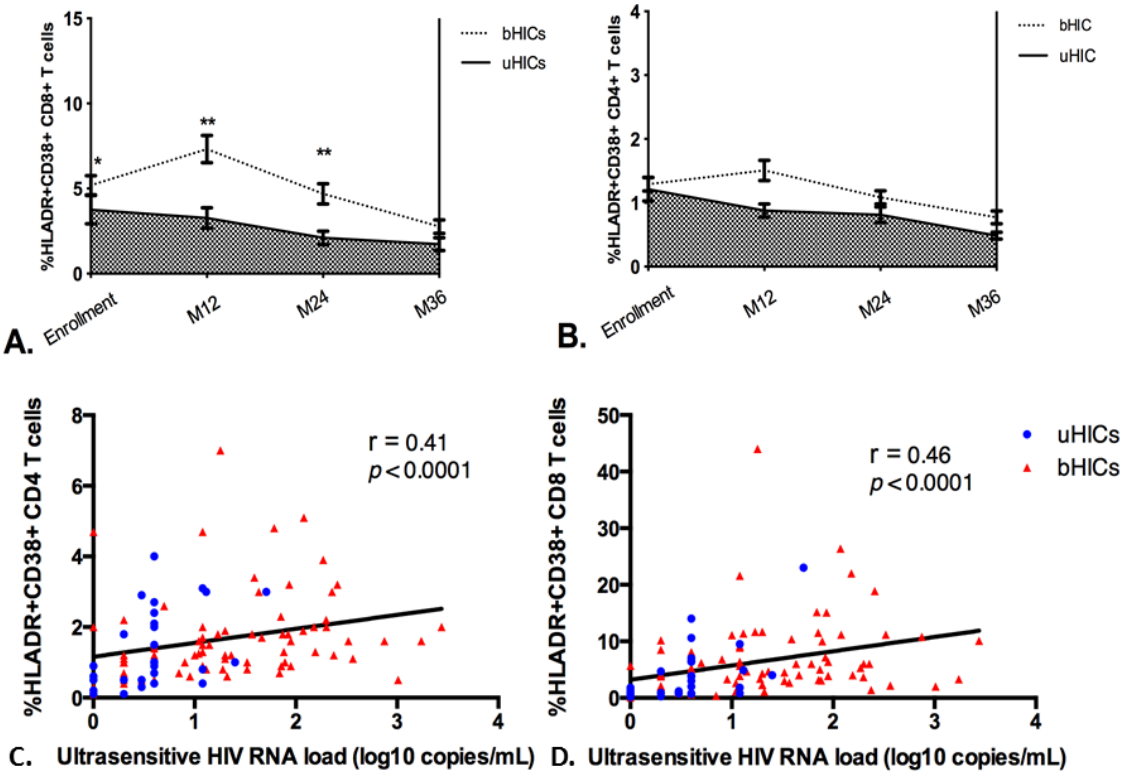
Accepted Manuscript

Figure 1



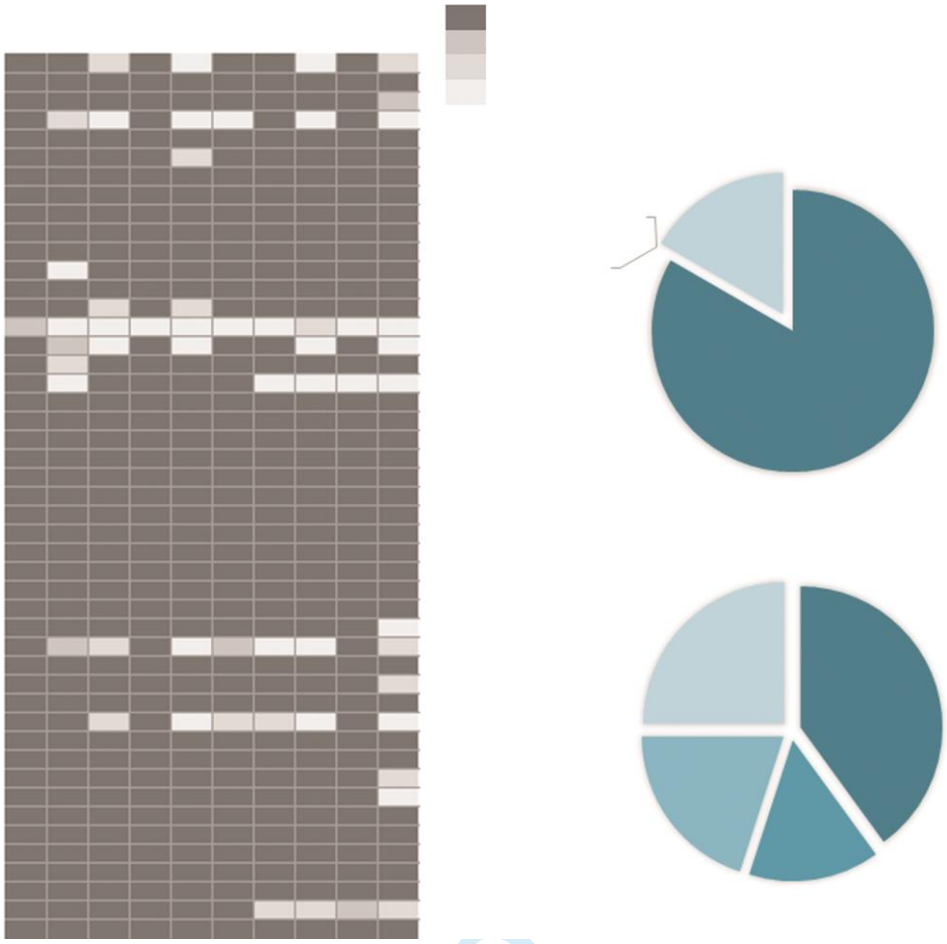
Accepted

Figure 2



Accepted

Figure 3



Accepted Manuscript