# HIV CONTROLLERS: A GENETICALLY DETERMINED OR INDUCIBLE PHENOTYPE?

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**Summary**

Less than 0.5% of HIV-1 infected patients maintain viral load below the threshold of detection by conventional assays for many years without antiretroviral therapy. These patients are known as HIV controllers (HICs) and offer a unique opportunity to explore and unravel efficient mechanisms of defense against HIV. An intense research effort has led to identify viral and host factors that contribute to the control of viral replication. Although the host factors appear to be the primary determinants for HIV control, different genomic, transcriptomic and immunological profiles have been described in HICs. This supports the existence of different mechanisms of control. We review here the most significant data in the field, including our own work, in the light of a crucial question: whether the ability to achieve and maintain the control of HIV replication is linked to genetic or immunological features specific to HIC or can be induced by vaccine or therapeutic approaches in the general population.

Keywords: HIV-1, HIV-controllers, Post-treatment controllers, antiviral responses, viral reservoirs

## Introduction

The pathogenesis of HIV infection in humans depends on a balance of viral and host factors. The fitness of the virus and the type of cell that it is able to infect may have an enormous impact on the rate of progression to disease (1-3). The virus relies on numerous cellular factors to complete its replication cycle, and it needs to altogether overcome intrinsic cell factors that are devised to hinder viral replication (4-6). Moreover, the host mobilizes a panoply of immune responses, both innate and adaptive, to counteract infection. A better understanding of the intimate interactions between the virus and the host will undoubtedly provide important clues in the search for an HIV vaccine and cure. Interestingly, in a few patients, the host factors appear to have prevailed over the virus, as these patients are able to naturally control viral replication, maintaining extremely low virus levels (7-11). These patients are known as HIV controllers (HICs) and offer a unique opportunity to explore and unveil efficient mechanisms of defense against HIV.

## Clinical characteristics

Transitory control of infection may occur in up to ~7% of all HIV-infected patients (12). However, in most of these patients, control of the infection does not last long and is lost within less than 12 months. In contrast, once control of viremia has been maintained for at least one year, the probability of these patients maintaining undetectable viral replication for numerous years is increased. Overall, the frequency of these long-term HIV controllers is low. Several epidemiological studies have estimated that these patients may represent around 0.5% of the total population of HIV-infected patients (9, 13, 14). The definition of HICs varies worldwide depending on the threshold of maximal viral replication that is used or

the length of control (15). In the French ANRS CO21 cohort, HICs are defined as patients naïve to antiretroviral therapy (except for transient treatments during pregnancy) who have been infected for at least five years and have maintained their five last viral loads below 400 HIV RNA copies per ml of plasma. The 400 RNA copy threshold was chosen for historical reasons, as this was the threshold of the first viral load tests largely available in French clinical centers in the 90s. Other cohorts, such as the international HIV controller consortium, have defined these patients as HIV-infected individuals who have maintained at least two consecutive plasma viral loads below 50 copies/ml within the last 12 months without therapy (10).

Patients who will become HICs typically have a non-symptomatic primary infection and have higher CD4+ T cell levels and lower plasma viral loads and reservoir levels in the blood compared to those who will not achieve control of infection (12, 16). This suggests that efficient mechanisms of control start acting soon after infection in most of these patients (9). However, the control of infection in some particular cases is a progressive phenomenon that may require several years to accomplish (9). What differentiates these late controllers from early controllers remains unknown, but comparative studies may provide interesting insights into the development and relative contribution of the different mechanisms that may participate in establishing control of infection in HICs. Little information is available regarding co-infections by other pathogens in HICs, but at least some of these patients appear to be able to control or even clear hepatitis C infection (17, 18).

Although HICs maintain a remarkable control of infection, blips in viral load levels have been observed for many of these individuals during long-term follow-up (13). Very low levels of plasma virus can be detected by ultrasensitive RT-PCR assays in HICs, revealing a persistence of viral replication despite maintaining viremia below the limit of detection by standard RT-PCR (19-21). Interestingly, these patients may have been able to control infection of the central nervous system even more effectively, as detection of viral replication is infrequent in cerebrospinal fluid from these patients, even when using ultrasensitive techniques (22, 23). Many HICs also experience a decline of their CD4+ T cell levels over time (13, 24). It is likely that the loss of CD4+ T cells in HICs is related to low-level viremia; we and others have recently reported that declining CD4+ T cell levels were observed in patients who had experienced blips in their viral loads or had higher low-level viremia (13, 21). In contrast, for some HICs, a viral blip has never been recorded over very long follow-up periods (13). These patients maintain stable CD4+ T cell levels, and many have undetectable viral loads even when using ultrasensitive techniques able to detect 1 RNA copy/ml of plasma (our unpublished observations). Interestingly, these HICs are close to healthy individuals from a transcriptomic point of view (25).

The slopes of CD4+ loss in HICs are typically very modest (13). However, AIDS-related events have been reported for a couple of HICs (24), and CD4+ T cell losses have prompted therapeutic intervention in a few others (26, 27). Although data on such patients and their clinical evolution are scarce, the reaction of HICs to antiretroviral treatment appears to be unequal. Some regain their CD4+ T cell counts, although their gains are smaller than those of patients with uncontrolled viremia who start therapy (26). Other patients do not increase

their CD4+ T cell numbers despite exhibiting a reduction in their low-level viremia upon therapy initiation (27). This would suggest that additional factors underlie the evolution of CD4+ T cell counts in HICs, and their identification may help to devise additional therapeutic approaches to implement in these HICs and other HIV-infected patients as a complement to antiretroviral therapy.

CD4+ T cell loss in HICs has been associated with elevated levels of T cell activation (24). Although lower compared to viremic patients, most HICs have abnormally high levels of T cell activation and inflammatory markers and signs of microbial translocation when compared to the healthy population or, for some markers, HIV-infected patients receiving antiretroviral therapy (24, 28, 29). Indeed, high levels of immune activation may well be as important a determinant of CD4+ T cell loss in HICs as they are in uncontrolled infection. However, high levels of immune activation are a rather common characteristic observed in HICs and do not entirely explain the more profound loss of CD4+ T cell counts in the rare cases presented above.

As in other HIV-infected patients, HICs have higher proportions of differentiated T cell subsets compared to healthy donors (30). Two recent reports suggest that HICs can regenerate T cells through optimal thymic output, homeostatic proliferation and preserved lymphopoiesis (30, 31). In contrast, HICs experiencing significant progression have reduced levels of hematopoietic progenitor cells (31), which likely compromises thymic function in these patients (30). Thus, there may be some cost for HICs to maintain an optimally tuned

immune response against HIV (see below), but the benefits overwhelm the disadvantages for the vast majority of HICs.

## Host-virus interactions involved in HIV control

### Viral determinants

The low levels of viremia in HICs are similar to those found in HAART-treated patients (19, 21, 32), and a median value of 2 copies/ml in a cohort of HICs has been reported (33). In contrast, HIV-1 reservoirs are much smaller in HICs than in patients on HAART (7, 34, 35). This discrepancy raises the question of whether plasma viruses in HICs originate from reactivations of this decreased latent reservoir or correspond to ongoing viral replication. Cross-sectional studies of plasma and cell-associated virus sequencing from HLA-B*57-positive HICs have shown discordance between *gag* plasma viral sequences and sequences from archived provirus in resting CD4+ T cells (32). All the amplified plasma viral clones had mutations in HLA-B*57-restricted epitopes, which is consistent with selective pressure from the CTL response. In contrast, almost all proviral clones were wild type, suggesting that the infecting founder viruses did not contain escape mutations. Strong evidence indicating that persistent viremia in HICs is sustained by continuous cycles of viral replication comes from longitudinal studies in which gene sequencing was performed in sequential samples. The analysis of *gag* and *nef* sequences from the plasma of HLA-B*57- and/or HLA-B*27-positive HICs showed a temporal evolution of viral genes (36, 37). Interestingly, genetic evolution was primarily restricted to the plasma viruses and was rare in cell-associated viral sequences. *Gag* and *nef* proviral clones appeared to be ancestral to the plasma clones and likely represent archived viruses. This suggests that viral reseeding and expansion of the

latent reservoir is not allowed by the very low rate of HIV-1 replication in the cellular compartments of HICs. While mutations found in HLA-B*57/5801- and HLA-B*27-restricted epitopes persisted over time, suggesting a continuous CTL-driven pressure, the majority of the evolution observed in the *gag* and *nef* genes in the patients included in these studies was driven by synonymous mutations and not by evolution in CTL-targeted epitopes (36). A lower rate of viral replication after an initial control of viral replication by immune responses may explain the lower frequency of nonsynonymous mutations and CTL escape variants in HICs compared to chronic viremic patients. Single-genome sequencing followed by a phylogenetic analysis of the *pro-rt* and *env* genes from plasma samples also showed that sequence divergence in these genes increases with time in many HICs (20). In this study, evolution of plasma viruses was found both in patients who did not have protective HLA alleles and in patients carrying the HLA-B*57/27 alleles, indicating ongoing virus replication despite the presence of these protective HLA alleles. However, in accordance with the results reported for *gag* sequences (36), significantly fewer mutations within known CTL epitopes in Pro and RT were found in HICs than in noncontroller patients (20).

Strong cellular response-driven immune selection has been associated with a reduced replication capacity of mutant viruses (38-40). Accordingly, chimeric HIV-1 viruses containing the *gag-pol, rev, vif,* and *env* genes isolated from the plasma of HICs showed reduced replicative capacity compared with viruses containing the corresponding genes isolated from patients with progressive disease (41, 42), (43, 44). In addition, the reduced viral fitness of *gag-protease* clones derived from HICs was correlated with mutations in epitopes restricted by protective HLA alleles (40, 42). A recent study documented a relative impairment of Nef functions in Nef clones isolated from HICs compared to those from chronic progressors (45). Noncanonical B57*-associated polymorphisms in CTL epitopes, different from the common

polymorphisms described in progressors, have been associated with Gag-Pol and Nef attenuations in HICs (40, 45). These results suggest that pressure by HLA-B57* CTLs selects for rare variants in HICs leading to lower viral replication capacity. This is well illustrated by a study that analyzed the common HLA-B57/B*5801 associated T242N mutation in the Gag TW10 epitope found in B57/B*5801-positive HICs and HIV progressors. Rare variants that reduced the viral replicative capacity were found exclusively in HICs within and flanking the TW10 epitope; these variants were recognized by variant-specific CTL responses (40). These studies support a reduction of viral fitness associated with CTL escape mutations in chronic infection. The open question is whether infection with a mutated attenuated virus may be at the origin of the control of viremia in HICs or whether viral attenuation is caused by the strong immune response that leads to the control of viremia. The evolution in HLA-B*57/B*27-restricted epitopes found in plasma viruses but not in the cell-associated viruses of HICs, as mentioned above, is in favor of the second hypothesis. Obvious genetic defects, such as deletions, insertions and stop codons, have not been found in viruses isolated from HICs, except in sporadic cases ((46) and references herein). However, one study has described an impaired replicative capacity of plasma gag-protease clones obtained during the acute/early phase of infection from patients who subsequently become controllers (47). In some cases, HICs were recipients of drug-resistant strains or of strains containing B57-associated escape mutations, suggesting the transmission of attenuated viruses; others carried HLA-protective alleles that could have forced the selection of escape mutations in early phases of infection. These data suggest that the characteristics of the infecting viruses and an early selection of fitness cost mutations by protective HLA alleles are conditions surrounding viral control in HICs. However, many HICs do not carry protective alleles, and mechanisms of viral control that are dependent on immune selection cannot be generalized

to all the patients. Moreover, all the studies performed with chimeric viruses focused on isolated genes introduced into a reference heterologous backbone strain and did not address the replicative potential of whole viral isolates. Therefore, if the weakened function of viral proteins appears to be frequent in HICs, this does not exclude the possibility that viruses infecting HICs can exhibit replicative fitness. Indeed, replication-competent viruses were isolated from the plasma of HICs from different cohorts, including the French ANRS cohort, and showed replication levels similar to those of reference HIV-1 strains (34, 48-50).

Overall, some important points can be inferred from these studies: infection with an attenuated/defective virus can make achieving control of viral replication easier in some HICs, but it alone does not explain the HIC phenotype. Indeed, control of infection has been reported in patients receiving virus from pairs with progressive infection, suggesting that host factors are the most important determinant for HIV control (51). When present, protective HLA alleles help to achieve and/or maintain control of viremia but still require the presence of other factors. Indeed, HICs and progressors carrying the same HLA allele display different viral polymorphisms and sequence evolution. In addition, different outcomes of transmitted viruses have been reported in HLA-B*57-positive HICs and progressors; viruses from progressors showed compensatory mutations and became progressively more fit over time, whereas viruses from HICs did not (52),(53).

**Genetic determinants of the innate and adaptive immune response**

A number of studies have attempted to understand the contribution of genetic factors and of immune responses to viral control. Genome wide association studies (GWAS) have located the genetic determinants with the strongest association with HIV-1 control in the HLA class I

region in the major histocompatibility complex (MHC) on chromosome 6 (54, 55). The single nucleotide polymorphisms (SNPs) with the highest significant association were found in close proximity to the HLA-B and HLA-C genes, which is consistent with earlier reports showing an association between specific HLA class I alleles and disease progression (56). The strongest association was found with a SNP located in the HCP5 gene that is in linkage disequilibrium with the HLA-B*5701 allele in whites and with HLA-B*5703 in African-Americans (57, 58). A deeper analysis by inferred sequencing of the HLA class I alleles in conjunction with GWAS showed that the specific amino acid composition in the HLA B peptide-binding groove underlies the associations between protection or disease progression and protective alleles such as B*27, B*57 and B*14 and deleterious alleles such as B*35, respectively (59). The second association with HIV-1 control was found with a SNP 35 kb upstream from the HLA-C gene (54, 59). This SNP was also associated with higher expression of HLA C alleles on the surface of cells (60). This effect on HLA C expression has been subsequently explained by a differential binding of microRNA Hsa-miR-148a to its target site within the 3'untranslated region of HLA-C, which leads to a specific post-transcriptional regulation of different HLA-C alleles (61).

It is important to note that the HLA genetic associations together explain 19% of the variance of host control (59), indicating that other factors are involved in determining the HIC phenotype. However, it is not totally surprising that GWAS have identified only the genetic determinants located in the MHC region, considering the low frequency of HICs (~ 0.5% of HIV-1-infected individuals) and their heterogeneity. Indeed, only common variants can be detected by linkage in a population, and standard GWAS do not have the power to detect rare variants (62).

The strong genetic associations in the MHC region point to the role of immune responses in HIV-1 control at least in some HICs. The associations between specific HLA class I alleles, HIV-1 control and, in particular, the impact of the peptide composition of the class I binding groove on viral peptide-binding capacity suggest a direct role for the specific cytotoxic T lymphocyte (CTL) response. However, HLA class I molecules are also ligands for NK cell killer cell immunoglobulin-like receptors (KIRs). Certain KIRs, namely KIR3DL1 and KIR3DS1, have been found to be associated with better control of HIV-1 when found in patients that also have HLA-B alleles with Bw4 epitope specificity (Bw4-80I) (63). In line with this observation, NK cells from individuals carrying KIR3DL1 receptor-HLA-Bw4 ligand pairs perform multiple functions in response to stimulation (64). The HLA-B alleles that have been associated with HIV-1 control (HLA-B*57/27/58) are of the Bw4-80I group, suggesting that the mechanisms of viral control may not only be due to CTL responses but also to the induction of an efficient NK cell response through their interaction with NK cell receptors.

However, data regarding the impact of NK cells on HIV-1 control in HICs are scarce. The combination of the KIR3DL1*h/*y genotype and HLA-B*57 has been associated with protection from HIV-1 disease progression (65). NK cells from subjects carrying the *h/*y+B*57 genotypes exhibit the highest polyfunctional potential, but this is dependent on cocarriage of the NK receptor and its HLA-Bw4*80I ligand (64). One study reported increased target cell-induced degranulation and cytokine secretion following IFNα stimulation in NK cells from HICs carrying the inhibitory KIR3DL1*h/*y receptor genotype and the corresponding HLA-Bw4*80I ligand (66). However, NK cells from HICs show a modest capacity to suppress viral replication in autologous CD4+ T cells *in vitro*, much lower than that mediated by CD8(+) T cells ((67), and our unpublished results).

**Adaptive responses**

Cytotoxic CD8+ T lymphocytes

In contrast to the NK response, the HIV-1-specific CD8+ T cell response in HICs has been the focus of intense investigation. Despite the extremely low level of viral antigen, many HICs, whom we have termed “strong responders”, present a high frequency of HIV-1-specific CD8+ T cells that is much higher than in HAART patients, despite having comparable viral loads (33, 49, 68). However, the magnitude of this response, measured by IFN gamma ELISPOT assay, is similar or even lower than in viremic progressors (10, 68), suggesting that viral control implies qualitative differences not revealed by this assay. Several phenotypic markers are differentially expressed on HIV-1-specific CD8+ T cells from HICs in comparison with viremic progressors, including markers of T cell activation/exhaustion, such as PD-1 and CD57 (69, 70). A peculiar trait of HIV-1-specific CD8+ T cells from HICs is the discordant expression of the activation markers HLA-DR and CD38. Whereas HIV-1-specific CD8+ T cells from viremic progressors express high levels of the two markers and those from HAART patients express low levels of both, HIC CD8+ T cells express high levels of HLA-DR and low levels of CD38 (68). This profile was previously described for the entire $CD8^+$ T cell population in asymptomatic HIV-infected patients with stable $CD4^+$ T cell counts (71). This differential expression might be linked to a superior capacity to respond to antigenic stimulation in the absence of nonspecific activation driven by the inflammatory context present in viremic patients. Accordingly, HLA-DR expression was correlated with the percentage of proliferating cells among HIV-specific $CD8^+$ T cells (68). A higher ability of HIV-specific CD8 T cells from HICs to proliferate in response to HIV-infected CD4+ T cells or HIV-1 peptides in comparison with viremic patients has been consistently found and associated in many studies with the protective HLA-B*57 allele (8, 68, 72). The level of polyfunctional HIV-specific CD8+ T cells,

defined by the capacity to exert multiple effector functions, including the secretion of several cytokines, such as IFN gamma, TNF-α, MIP-1β, CD107a and IL-2, following antigenic stimulation, is inversely correlated with viral load (73). A polyfunctional response is found in HICs and is scarce or absent in viremic progressors, most likely as a consequence of persistent exposure to antigen during viremic infection (73, 74). The functional property that most clearly differentiates the HIV-specific CD8+ T cells of HICs from those of viremic progressors and HAART-treated patients is their capacity to directly kill infected cells *in vitro*. HIV-specific CD8+ T cells from HICs have been shown to effectively suppress viral replication in autologous CD4+ T cells by eliminating HIV-infected cells without the need for exogenous stimulation (68). This suppressive capacity is not observed in either viremic progressors or in HAART-treated patients and has been associated with an effective loading of perforin and granzyme B in CD8+ T cells from controllers upon contact with HIV-infected cells (75, 76). Of note, whereas more efficient loading of lytic granules has been documented in CD8+ T cells after 6 days exposure to antigen (76), the killing of infected cells is detected a few hours after adding CD8+ T cells to infected CD4+ T cells (68). This may be related to the ability of CD8+ T cells from HICs to rapidly upregulate perforin and granzyme B following exposure to antigen, an ability that has been associated with high levels of expression of the T-box transcription factor T-bet after the *in vitro* expansion of HIV-specific CD8+ T cells (77). The rapid elimination of infected cell targets by *ex vivo* CD8+ T cells implies the presence of CTLs with immediate effector functions. Consistent with these results, differentiated effector CD8+ T cells directed against HIV-1 are found more frequently in HICs compared to HIV-1 progressors (68, 78).

CD8+ T cell suppressive capacity in HICs is highly correlated with the anti-Gag response, not with Nef- or Env-specific responses (49, 50, 79, 80), which is consistent with earlier studies

reporting an association between the anti-Gag CD8+ T cell response and viral control (81, 82). A direct contribution to the control of the viral reservoir by the Gag-specific CD8+ T cell response in HICs is suggested by their association with a low HIV reservoir in the central memory CD4+ T cells of HICs carrying HLA-B* 57/27 alleles (83).

An increased magnitude and polyfunctionality of CD8+ T cell responses to HIV Gag has also been found in the rectal mucosae of HICs and was more strongly associated with controller status than peripheral blood responses (84, 85). It has been suggested that a superior T cell response in the intestinal mucosae may contribute to limit CD4+ T cell depletion and preserve mucosal integrity (84). The superior antiviral activity of Gag-specific CTLs may be related not only to their lytic function but also to the selection of immune escape variants with lower replication capacity (40-42, 86, 87), as discussed above.

Several studies suggest that Gag-specific CTLs from HICs mediate effective viral control because of their higher functional avidity, i.e., the ability to react to lower antigen concentrations and to recognize more epitope variants than those from HIV progressors (88, 89). Accordingly, control of HIV infection has been associated with the selection of HLA-B*27-restricted KK10 epitope-specific CD8+ T cell clonotypes with high avidity, polyfunctionality and suppressive capacity (90). A major step in our understanding of the mechanisms underlying the superior quality of the CD8+ T cell response in HICs came from two recent studies that compared $CD8^+$ T cell responses to Gag-immunodominant viral epitopes in HICs and chronic progressors bearing HLA-B*27 or B*57 (91, 92). The ability of $CD8^+$ T cells targeted to the same epitopes to inhibit viral replication was higher in HICs than in HIV progressors and was associated with a different selection of epitope-specific TCR clonotypes during infection. The TCR clonotypes of HICs displayed a better capacity and higher avidity to cross-recognize naturally occurring viral variants (92) and upregulate

perforin and granzyme B more than the TCR clonotypes of progressors (91). Interestingly, the selection of these clonotypes occurs very rapidly after infection in controller patients and in the context of low-level viremia (92). These studies provide an explanation for why only a few individuals bearing the HLA-B* 27 and B*57 alleles control HIV-1 infection and links the protective effect of these alleles to peculiar TCR rearrangements that lead to the selection of the most effective clonotypes.

How favorable TCR clonotypes are selected in HICs remains unknown. The peculiar amino acid composition of the protective HLA B peptide-binding groove (59) may favor an optimal presentation of the viral peptide. It has also reported that the myeloid dendritic cells of HICs efficiently generate T cell responses and that this capacity is associated with increased expression of the leukocyte immunoglobulin-like receptors LILRB1 and LILRB3 (93). However, additional studies will be needed to clarify these issues.

Polyfunctional and effective HIV-specific CD8+ T cells in strong-responder controllers may result from the maintenance of an efficient response developed during the primary infection that is lost in HIV progressors due to immune activation-driven T cell exhaustion. We addressed this question in a study on a large number of patients during primary infection who were included in a clinical assay (Optiprim) before the onset of HAART (94). We found that most patients exhibited high frequencies of polyfunctional HIV-specific CD8+ T cells, but these cells lacked strong HIV-suppressive capacities *ex vivo*. This indicates that HIV-suppressive capacity is not a common characteristic of the CD8+ T cell response during primary infection and suggests that it is an intrinsic feature found in HICs. This also implies that this quality of the T cell response is a cause and not a consequence of viral control.

If the presence of protective alleles and an effective CTL response certainly play a major role in viral control in many HICs, particular MHC alleles or a strong CD8+ T cell response are not necessary to achieve controller status. Indeed, as discussed above, HICs are a heterogeneous population: many HICs lack protective HLA alleles and/or have weak HIV-specific $CD8^+$ T-cell responses (10, 49, 95). A potential role of CD8+ T cells in viral control that is also observed in HICs who have a low frequency of specific CD8+ T cells has been suggested by recent reports showing that specific CD8+ T cells with viral suppressive capacity can be expanded upon stimulation with Gag peptides, consistent with the presence of HIV-specific CD8+ T cells with a memory phenotype (96, 97). In an *in vitro* suppressive assay, sorted effector memory and terminal effector HIV-specific CD8+ T cells exhibited the strongest suppressive capacity in the first days of CD8+/CD4+ T cell co-cultures. However, central memory CD8 T cells that expanded after 5 days of incubation with antigen also acquired an effective suppressive capacity (96). If these studies suggest that the presence of a few specific central memory CD8+ T cells may be sufficient to maintain the control of eventual reactivations of HIV-1 replication in weak-controller HICs, this conclusion should be moderated by the following considerations: the study of Buckheit included only HLA-B* 57-positive patients and therefore may concern qualitative features of the CTL response linked to this protective allele; other studies have reported that pre-stimulation of CD8+ T cells from HAART patients with HIV antigen also leads to the efficient killing of infected cells and that the primary difference with CD8+ T cells from HICs is their rapid viral suppressive activity (98).

Regulatory T cells

Several reports, if not all, have reported that the frequency of regulatory T cells (Tregs) is not increased in HICs and is similar or lower compared to uninfected individuals (99-102). This

may contribute to the maintenance of high-quality HIV-1-specific CD8+ T cell responses in HICs. The cost may be the persistent immune activation found in most HICs despite controlled viremia (see previous section) (24).

Humoral response

As in the case of the CD8+ T cell response, HICs also have a heterogeneous humoral response. As could be expected due to their low levels of viremia, neutralizing antibodies are present at low levels in HICs and are unlikely to play a major role in the control of infection (10, 103). In contrast, ADCC potential, both related to the quality of non-neutralizing antibodies and the cell surface expression of FCγ receptors, appears to be greater in controllers (103-105). Nevertheless, the weakest antibody levels are found among HICs with the best control of infection (no viral blips, no CD4+ T cell loss), and weakly reactive/partial western blots have been reported for some of these HICs (106).

CD4+ T cell response

CD4+ T cell help is required to sustain an effective CD8+ T cell response in chronic viral infections, and numerous studies have addressed the characteristics and the potential anti-viral role of CD4+ T cells in HICs. GWA and transcriptomic analyses of CD4+ T cells, comparing elite controllers with patients successfully treated with HAART and healthy donors, did not find a transcriptome profile characteristic of viral control (107). This study demonstrated that viral replication was the major driver of RNA expression changes in HIV-1-infected individuals. Consistent with that report, another study focusing on HLA-DR$^-$ CD4+ T cell subsets showed that the transcriptional profiles of most elite controllers were similar to those of successfully HAART-treated patients but different from those of HIV-1-negative

persons (25). Although it was not possible to identify transcriptional patterns specific of HICs, phenotypic and functional profiles of HIV-specific CD4+ T cells indicated a higher quality and superior survival in HICs compared to viremic or HAART-controlled patients, as described for CD8+ T cells. A comparison between HICs and HAART-treated patients with controlled viremia showed equivalent levels of expression of activation markers on the CD4+ T cells of HICs, but they were lower compared to the CD4+ T cells of viremic patients and higher compared to those of healthy donors (24, 29, 108, 109). The CD4+ T cells of HICs have lower levels of expression of the negative immunoregulatory molecule cytotoxic-T-lymphocyte-associated antigen 4 (CTLA-4) compared to those of HAART patients, which is consistent with the increased functionality of these cells (110). In contrast to viremic patients, the CD4+ T cells from HICs maintain the ability to secrete IL-2 and proliferate upon HIV-1 Gag stimulation, which is associated with a preserved central memory population (29, 111). Compared to viremic patients and healthy individuals, a lower level of FoxO3a-mediated pro-apoptotic transcriptional activity in memory T cells from HICs has been proposed as a molecular basis for the preservation of memory CD4+ T cells in HICs (112). Memory T cells from HICs show high avidity for immunodominant Gag peptides and are capable of maintaining robust responses despite the presence of only a low level of viral antigen (113). Like CD8+ T cells, CD4+ T cells from HICs can secrete several cytokines/chemokines simultaneously in response to antigen (29, 108). Polyfunctional CD4+ T cell responses associated with the HLA-DRB1*13 and HLA-DQB1*06 alleles were also found in the gut mucosa of HICs (114). Consistent with high levels of IL-$2^+$/IFN-$\gamma^+$ production by HIV-specific CD4+ T cells, a high frequency of CD4+ Th1 effectors in response to immunodominant Gag peptides has been reported in HICs (109). Taken together, these data are consistent with the presence of HIV-specific, long-lived, highly functional memory CD4+ T

cells with the capacity to be activated and rapidly proliferate in response to viral reactivation in HICs. These CD4+ T cells would provide a helper function for driving and sustaining the antiviral CD8+ T cell response.

However, memory CD4+ T cells are the preferred target of HIV-1, and infected central and transitional memory CD4+ T cells have been reported to be the major cellular compartments of the latent HIV reservoir (115).

**Intrinsic immunity**

Some studies have reported that CD4+ T cells from HICs are relatively resistant to HIV-1 infection *in vitro* compared to CD4+ T cells from healthy donors or viremic patients (116-118). Interestingly, the decreased susceptibility of CD4+ T cells correlates with the level of viral DNA that these CD4+ T cells carry *in vivo*, suggesting that it may contribute to the decrease of the viral reservoir in HICs (117). Decreased permissiveness to HIV-1 replication was also found in the monocyte-derived macrophages of HICs and was related to the inhibition of the accumulation of viral reverse transcripts in both CD4+ T cells and macrophages (117). An additional inhibition at the level of HIV-1 transcription has been described in CD4+ T cells (116). Viral restriction does not depend on the upregulation of known retrovirus restriction factors, including *TRIM5α*, *APOBEC3 family*, *BST2/Tetherin*, and accordingly, transcriptomic data show that the expression of these genes is driven by an increasing viral load (107) and our unpublished results). We and others have reported that CD4+ T cells from HICs have increased *ex vivo* expression of the CDK inhibitor p21 compared to uninfected individuals (116, 117). p21 has been previously shown to inhibit the preintegrative steps of HIV-1 replication *in vitro* in macrophages and hematopoietic stem cells (119, 120). One study demonstrated that p21 overexpression was involved in the

restriction of HIV-1 replication in activated CD4+ T cells (116); by contrast, we did not find a direct role for p21 in HIV-1 restriction in CD4+ T cells or macrophages from HICs (117). The permissiveness of target cells to HIV-1 remains a debated question because other authors have found that CD4+ T cells from HICs and uninfected individuals have similar susceptibility to HIV-1 (121). Interestingly, however, the study by O'Connell reported that CD4+ T cells from HICs produced less virus per infected cell than CD4+ T cells from viremic progressors (121), suggesting a further defect that could affect viral spread. A study conducted in a few HICs reported hypermethylation of the HIV-1 proviral 5'-LTR in comparison with HIV progressors (122), suggesting that epigenetic modifications may contribute to the control of viral replication. This hypothesis is consistent with the observation that despite an effective control of viremia and an extremely low cell-associated HIV-1 DNA level, infecting virus can be isolated from CD4+ T cells from many HICs upon activation with mitogens, cytokines or TCR stimulation (34, 48-50), suggesting that viral expression from latently infected cells may be repressed *in vivo*.

In conclusion, virologic and/or genetic factors likely account for viral control in some HICs. Adaptive immune responses, specifically polyfunctional $CD8^+$ and/or $CD4^+$ T-cell responses, may limit viral replication, particularly in strong-responder HICs. HICs lacking protective MHC alleles and/or having weak T cell responses may rely on other mechanisms, such as the cellular restriction of viral replication and/or innate immune responses that have not been clearly identified.

**Post-treatment controllers**

HICs are accepted as an appealing example of what a functional HIV cure may represent (123). Other than to better understand how they control infection, the challenge now is to devise ways to induce a similar phenotype of control in a larger group of patients and irrespective of their MHC background. Some HIV patients diagnosed early in primary infection who start antiretroviral treatment almost immediately and maintain treatment for long periods of time (more than 1 year) are able to durably control viremia after treatment discontinuation (124-127). Because of the rapid therapeutic initiation, one of the difficulties when studying these post-treatment controllers (PTCs) is to exclude the possibility that they may have also been able to control infection without any intervention. Although data on these patients are still scarce, many differences have been observed between PTCs and natural HICs. Whereas natural control of viremia is generally associated with non-symptomatic primary infection, lower viral loads and high CD4+ T cell numbers (12, 16), most PTCs have a highly symptomatic primary infection with high viral loads and low CD4+ T cell numbers at levels comparable to patients who will not control the infection (127). Moreover, protective HLA alleles are not overrepresented among PTCs, who show a significant frequency of some HLAs that are rarely found in HICs and are associated with higher viral loads (127). Overall, PTCs have lower levels of T cell activation and much weaker and less efficient CD8+ T cell responses than HICs. Additionally, the probability of controlling infection for at least one year after the interruption of treatment initiated close to the primary infection and maintained for at least one year is estimated to be 8-15% (124-127). This estimation is much higher than that for > 1 year of natural control of infection in patients diagnosed during the primary infection (12, 16). There are some cases in which PTC status was achieved after the interruption of an antiretroviral treatment initiated (in the context of detectable viremia) during chronic infection (128), but these cases are much more

infrequent. Therefore, early treatment appears to enhance the conditions that allow control of infection in some patients. Early treatment initiation limits the establishment of viral reservoirs (129) and the diversity of the virus (130), which may be easier to control later by undamaged immune responses (131-133). However, it is still unknown why only a fraction of patients treated early are able to control infection after treatment cessation. The precocity and duration of the treatment and the decrease of viral reservoirs to extremely low levels at the time of treatment interruption distinguished PTCs from non-controllers in two studies (124, 125). Similarly, the 14 PTCs described in the VISCONTI study, who have been controlling infection off therapy for a median of 7.5 years, initiated their treatment approximately 35 days after their presumed infection and were treated for a median of three years before interrupting treatment (127). The PTCs from the VISCONTI study also have very low viral reservoirs, similar to those of HICs, in which there is a small contribution of long-lived cells. Interestingly, these viral reservoirs have appeared to shrink in some PTCs in the absence of therapy (127). Nevertheless, further studies will be necessary to understand the factors allowing the control of infection in these appealing patients.

## Conclusions

From this review, it appears clear that HICs are not a homogeneous population; they can be distinguished by specific transcriptomic, genomic and immunological profiles. They are heterogeneous in terms of the viral loads determined by ultrasensitive single-copy PCR assays, HLA class I alleles, the frequency and quality of CD8+ and CD4+ T cell responses and levels of immune activation. It is yet not possible to associate a multiparametric profile that distinguishes different subpopulations of HICs. It is possible that the key parameters that determine eventual viral control may only be detectable early in infection and have thus

been partially missed in recent studies. Indeed, as mentioned, viral control is achieved early in infection in most patients. It is urgent to perform longitudinal studies on cohorts of acutely infected patients to identify viral and host factors that determine viral control. However, the data suggest that some HICs maintain high CD4+ T cell counts and transcriptional profiles and levels of immune activation similar to those in non-infected individuals. A specific effort should be dedicated to perform a more in-depth characterization of these individuals who may provide the most significant keys to understanding the mechanisms of viral control and to evaluate whether it is possible to induce viral control using preventive or therapeutic strategies. Many questions remain about the relative contribution of viral factors, innate host mechanisms of defense and specific anti-HIV T cell responses in the control of viral replication and the containment of viral reservoirs. One critical question is what viral reservoirs in the blood and tissue compartments fuel low-level viral replication in most HICs. Recently, it has been reported that the CD4+ T follicular helper (TFH) cell subset is expanded in lymph node germinal centers during HIV and SIV infection (134, 135), (136, 137). Replication-competent HIV was isolated from TFH cells not only from patients with high viremia but also from patients with low viremia (< 1000 copies/ml) after *in vitro* activation (137), suggesting that they may also be a source of viral replication in HICs. It would be informative to explore the frequency of HIV-infected TFH cells in the lymphatic tissues of HICs and their permissiveness to HIV-1 replication.

In contrast with HIC, who maintain a functional immune system because of a tight control of HIV replication, a natural control of pathogenesis in SIV-infected African monkeys is achieved in spite of high levels of virus replication. Sooty Mangabeys and African Green Monkeys, the best studied models of apathogenic infection, are protected against disease mainly because

of their capacity to rapidly down-regulate innate immune responses, including type I interferon production and IFN-related genes activation, after the burst that follows acute infection. The absence of chronic immune activation preserves lymphoid tissue integrity and immune functions, including the memory T cell pool. A thorough discussion on the mechanisms of control of viral pathogenesis in SIV-infected African monkeys can be found in (138).

As for the mechanisms of viral control in HIC, the data reviewed here support a complex model that involves different components of the immune system as well as those of the infecting virus (Figure 1). The different mechanisms may act together, or a specific mechanism may prevail in different individuals. It could be speculated that at the moment of viral transmission, an attenuated virus may result in slower replication kinetics and viral spread in some individuals. This would allow a better preservation of the immune system and the development of an adequate innate and specific cell response. In other individuals infected with fit viruses, either a lower permissiveness of the target cells or a rapid down-regulation of immune activation may hinder viral replication and the establishment of reservoirs. Efficient innate antiviral responses may also contribute to early viral containment. It is possible that in the case of PTCs, treatment during acute infection may reach the same goal by reducing the early dynamics of viral replication and limiting viral reservoir formation. In HICs, the genetic HLA background and/or unknown factors regulating T cell responses, including the selection of high CD8+ T cell receptor avidity, may take over during a second phase to maintain control of viral replication following the earliest responses in individuals who experience viral blips. Studies on HICs have highlighted the role of effective CTL responses, specifically those directed to HIV-1-Gag epitopes, in viral control. As discussed above, this CTL functional capacity can predict effective antiviral function and is measured by

*in vitro* suppressive assays. The protective role for CTLs *in vivo* has been recently supported by vaccine studies in a rhesus macaque model. The protective capacity of MHC-driven CTL responses has been demonstrated by the vaccination of Indian rhesus macaques that express the protective MHC Mamu-B*08 allele with Mamu-B*08-restricted CD8+ T-cell epitopes (139). The vaccinated animals controlled the replication of a pathogenic SIV clone after challenge. Perhaps even more promising, because the protection was not directly associated with the MHC background, the induction of SIV-specific effector memory T cells by vaccination with rhesus cytomegalovirus vectors efficiently controlled viral replication in macaques following mucosal challenge (140).

Although much work remains to be done to translate these vaccine approaches from the monkey model to humans, these data, together with the promising results arisen from the PTC studies, nurture hope that induction of HIV control may be possible, well beyond the small population of HIV controllers, in a not so far future.

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**Figure legends**

**Figure 1A**

In the acute phase of infection of the majority of individuals, HIV-1 exponentially replicates in target cells, including CD4+ T cells and monocytes/macrophages, reaching levels of several million copies of plasma viral RNA/ml. Viral load then declines with the development of host immune response and reaches a steady state that is a predictor of long-term disease progression. A massive destruction of CD4+ T lymphocytes occurs in the acute/early phase, especially within the lymphoid tissues of the gut. In the meantime, permanent viral reservoirs are established in the tissues. A dramatic cytokine cascade is activated in the acute phase, along with an abnormal activation of all the compartments of immune system, including NK cells, B and T lymphocytes. The levels of immune activation strongly correlate with the progression of disease. The impairment of HIV-1-specific CD4 T and CD8 T cell functions occurs early in acute infection and the gradual exhaustion of HIV-specific T cell response leads then to the loss of the control of viral replication at later times.

**Figure 1B**

In HIC, during acute infection, several factors may contribute, together or separately, to reduce the dynamics of HIV replication: the transmission of an attenuated virus, a lower permissiveness of the target cells (CD4+ T cells, dendritic cells and macrophages), efficient innate antiviral responses and a rapid down regulation of immune activation. This would limit the size of viral reservoirs and allow a better preservation of the immune system. A favorable genetic HLA background and the selection of high avidity CD8+ T cell receptor may then contribute to suppress viral replication and control viral blips in many HICs. Other still unknown factors may act to maintain viral control in other HIC.