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“HIV controllers” (HICs) are rare individuals in whom HIV-1 plasma viral load remains undetectable without antiretroviral treatment. This spontaneous viral control in HICs is usually associated to strong functional HIV-specific CD8+ T cell responses. Accordingly, we have recently shown that CD8+ T cells from HICs strongly suppress ex vivo HIV-1 infection of autologous CD4+ T cells, suggesting a crucial role of this response in vivo. Knowledge of the mechanisms underlying the CD8+ T cell antiviral activity might help to develop effective T cell-based vaccines. In the present work, we further characterized the HIV-suppressive capacity of CD8+ T cells in 19 HICs. CD8+ T cells from 14 of the 19 HICs showed strong HIV-suppressive capacity ex vivo. This capacity was stable over time and was partially effective even on other primate lentiviruses. HIV-suppressive capacity of CD8+ T cells correlated strongly with the frequency of specific HIV-CD8+ T cells, and in particular of Gag-specific CD8+ T cells. We also identified five HICs who had weak HIV-suppressive CD8+ T cell capacities and HIV-specific CD8+ T cell responses. Among these five HICs, at least three had highly in vitro replicative viruses, suggesting that the control of viremia in these patients is not due to replication-defective viruses. These results, on the one hand, suggest the importance of Gag responses in the antiviral potency of CD8+ T cells from HICs and, on the other hand, propose that other host mechanisms may contribute to restraining HIV infection in HICs.


Rare individuals called “HIV controllers” (HICs) spontaneously and durably control HIV infection in the absence of therapy, possibly illustrating what truly effective CD8+ T cell responses can achieve (15, 16). HICs have extremely low and stable amounts of viral DNA in their PBMC (17) and undetectable plasma viral load (18). The protective HLA alleles B27 and B57 are overrepresented among these individuals (4, 6, 19–21). Despite very low levels of Ag in blood (22), most but not all HICs have high frequencies of HIV-specific CD8+ T cells that preferentially target the viral Gag protein (20, 23, 24). Studies of CD8+ T cell responses in HICs have revealed important characteristics of functional HIV-specific CD8+ T cells in HIV infection. Contrary to cells from viremic individuals, HIV-specific CD8+ T cells from HICs can, upon stimulation with their cognate Ag, proliferate and generate a multifunctional response that includes perforin expression, degranulation, and chemokine/cytokine secretion (25–27). This could be related to a peculiarity of activation phenotype of these cells (21) and to constitutive telomerase activity that protects them against senescence (28). However, how much of this is the cause and how much the consequence of viral control and low-level immune activation remains to be determined. We have recently shown that CD8+ T cells from most HICs are endowed with a striking capacity to suppress HIV infection ex vivo (21), a property that is likely to be relevant in vivo. To further characterize this HIV-suppressive activity we extended our analysis to a larger

*Abbreviations used in this paper: HIC, HIV controller; moi, multiplicity of infection; PBMC, peripheral blood mononuclear cell; SFC, spot-forming cell.
group of 19 HICs and evaluated the relationship between this activity and HIV-specific CD8+ T cell responses.

Materials and Methods

Study subjects

Nineteen patients diagnosed with HIV-1 infection at least 10 years previously who had never received antiretroviral treatment and in whom > 90% of plasma HIV RNA assays gave values <400 copies/ml were studied (Table I): 8 have been described elsewhere (21), and 11 were newly recruited from the ANRS EP36 national monitoring program on HIV controllers. The subjects were serologically HLA typed by complement-mediated lymphocytotoxicity testing (InGen One Lambda). All had very weak and stable DNA load (Table I).

All of the subjects gave their written informed consent.

HIV DNA quantification

Total DNA was extracted from whole blood with QIAamp DNA minikits (Qiagen), according to the manufacturer’s instructions. HIV-1 DNA was then quantified by real-time PCR (LTR amplification; Agence Nationale de Médecine Infectieuse; www.hiv.lanl.gov/content/immunology/index.html). These peptides were synthesized by Neosystem and used at a final concentration of 2 μM. CD4+ T cells were reactivated on day 10 with CD8-depleted PHA-preactivated allogeneic PBMC, PHA, and IL-2. Virus-containing supernatants from CD8+ T cells were reactivated on day 10 with CD8-depleted PHA-preactivated allogeneic PBMC, PHA, and IL-2.

Infectivity assays were conducted in the presence of 100 IU/ml IL-2. We have previously shown that the presence of this cytokine during the infection assays did not affect the suppressive capacity of unstimulated CD8+ T cells (21).

Intracellular p24 assay

Activated CD4+ lymphocytes (5 × 10^6) were superinfected with HIV-1 BaL (R5) as described above. Various dilutions of virus (moi of 10^-5 to 10^-6) were used in parallel to obtain similar levels of infection in each individual/experiment. CD4+ T cells were culture in the presence of allosine of unstimulated CD8+ T cells. CD4+ T cells were reactivated on day 10 with CD8-depleted PHA-preactivated allogeneic PBMC, PHA, and IL-2. Virus-containing supernatants from CD4+ T cell cultures were titrated on mixed PHA-activated CD4+ T cells from two blood donors.

ELISPOT assay

IFN-γ secretion by HIV-specific CD8+ T cells was quantified ex vivo with an ELISPOT assay using appropriate stimuli (32). We used a set of 124 peptides corresponding to known optimal CTL epitopes derived from the HIV-1 Env, Gag, Pol, and Nef proteins (National Institutes of Health HIV Molecular Immunology Database; www.hiv.lanl.gov/content/immunology/index.html). These peptides were synthesized by Neosystem and used at a final concentration of 2 μg/ml. For each subject, optimal peptides were tested depending on the results of LTA typing with an average of 36 ± 9 peptides tested per subject. IFN-γ spot-forming cells (SFCs) were counted with a KS-ELISPOT system (Carl Zeiss Vision) and expressed as SFCs/cell (median CD4 count /10^3 cells).

Depletion of HIV-specific CD8+ T cells

Depletion of CD8+ T cells producing IFN-γ upon stimulation with HIV peptides was performed with an IFN-γ secretion assay enrichment kit.
Mitlenyi Biotec) as recommended by the manufacturer. Briefly, purified CD8^+^ T cells were stimulated for 6 h with appropriate pools of specific HIV peptides. Subsequently, the cells were labeled (5 min at 4°C) with an IFN-γ/catch reagent that attached to the cell surface of all leukocytes. The cells were then incubated for 45 min at 37°C to allow IFN-γ secretion. The secreted IFN-γ was captured by the IFN-γ/catch reagent on the positive, secreting cells. These cells were subsequently labeled with a second IFN-γ-specific Ab conjugated to R-PE. The IFN-γ-secreting cells were magnetically labeled with anti-PE magnetic beads and depleted by magnetic field separation. Purity of the depleted fractions was evaluated by flow cytometry.

Antibodies

The following Abs were used: CD8-ECD or -PC5 (clone B9.11), CD3-PC5 (UCHT1), CD45RO-ECD (UCHL1), HLA-DR-ECD (Immu-357), and CD38-FITC (T16), all from Beckman Coulter; and CD27-FITC (M-T271) from BD Biosciences.

Pentamer staining and phenotyping

HIV-specific CD8^+^ T cells were identified by using soluble PE- or allophycocyanin-labeled peptide-HLA class 1 multimers (Proimmune; Beckman Coulter Immunomics). The following epitopes were used: the HLA-A*0201-restricted peptide ligand KRWIILGLNK (Gag 263–272), and the HLA-B*5701-restricted peptide ligands KAFSPEVIPMF (Gag 162–172), TSTLQEQIGW (Gag 240–249), and QASQDVKNW (Gag 308–316). PBMC were incubated with pentamers (1 g/ml) for 30 min and then with relevant Abs for 15 min. Cells were washed in Cell Wash (BD Biosciences) plus 1% BSA, incubated for 10 min with FACS lysing solution (BD Biosciences). After washing, cells were fixed in 1% paraformaldehyde for flow cytometry with an Epics XL (Beckman Coulter) or a FACSCanto flow cytometer (BD Biosciences) and analyzed with RXP software (Beckman Coulter).

Proliferation assay

The proliferative capacity of HIV-specific CD8^+^ T cells was evaluated by flow cytometry. PBMC were stained with 0.35 M CFSE (Molecular Probes) for 10 min at 37°C, and, after washing, they were stimulated for 5 days with 2 µg/ml peptide or medium alone. After labeling with pentamer, anti-CD8, and anti-CD3 Abs, PBMC were fixed in 1% paraformaldehyde for flow cytometry as described above.

Statistical analyses

All values throughout the text are means ± SD. Values of p were calculated with the rank sum test. Correlations were identified by simple linear regression analysis and Spearman’s rank correlation test. SigmaStat 3.5 software was used (Systat Software).
**Results**

*Unstimulated CD8\(^+\)* T cells from most HICs have strong HIV-suppressive capacity

In a previous study we found that undetectable viremia in 9 out of 10 HICs was associated with a remarkably strong capacity of their circulating CD8\(^+\) T cells to control in vitro HIV-1 infection of autologous CD4\(^+\) T cells (21). To extend this observation, we used the same viral suppression assay to assess the *ex vivo* anti-HIV capacity of CD8\(^+\) T cells from 19 HICs, 11 of whom were newly recruited for this study and 8 were retested (Table I). A marked reduction in HIV-1 infection (*ex vivo*) was readily detected in the supernatants of purified CD4\(^+\) T cells from all 19 HICs after PHA activation and challenge with HIV-1 BaL (Fig. 1A). A marked reduction in HIV-1 infection (*ex vivo*) was generally observed when autologous unstimulated CD8\(^+\) T cells from HICs were added to the culture (Fig. 1A). The associated CD8\(^+\) T cell-mediated decrease in the level of HIV proteins was due to the absence of infected CD4\(^+\) T cells in the coculture (Fig. 1B). As a whole, the HIV-suppressive capacity of CD8\(^+\) T cells from HICs (2.79 ± 1.31 log p24 decrease, CD8/CD4 vs CD4) was much stronger than that of cells both from viremic individuals (0.82 ± 0.53 log p24 decrease, CD8/CD4 vs CD4) and autologous CD8\(^+\) T cells from weak responders (0.26 ± 0.63 log p24 decrease, CD8/CD4 vs CD4) (Fig. 1C). In particular, CD8\(^+\) T cells from 14 of the 19 HICs suppressed HIV far more strongly (log p24 decrease >2) than did cells from both viremic and unstimulated individuals (log p24 decrease <2) (Fig. 1C, D). These subjects are referred to below as strong responder HICs. Longitudinal analysis (>12 mo) of CD8\(^+\) T cell antiviral activity in five strong responder HICs included in our previous study suggested that this HIV-suppressive capacity is a stable characteristic (Table II). In contrast, here we identified five “weak responder” HICs (Table I) whose CD8\(^+\) T cells could not efficiently control HIV infection of autologous CD4\(^+\) T cells (log p24 decrease <2) (Fig. 1, B and C). The HIV-suppressive capacity of these subjects’ CD8\(^+\) T cells was not stronger than that of viremic or HAART-treated patients (Fig. 1C). We have reported that susceptibility of CD4\(^+\) T cells from HICs to *in vitro* HIV infection was not different than that of cells from healthy blood donors (21), and no significant differences were found either between weak responder and strong responder HICs (*p* = 0.331) (Fig. 1A).

To determine whether the weak HIV-suppressive activity observed in certain HICs was due to our use of a laboratory-adapted HIV strain, we analyzed the capacity of nonstimulated CD8\(^+\) T cells from weak responders A13 and A19 and from strong responder A21 to suppress superinfection of their own CD4\(^+\) T cells by autologous viruses previously obtained in primary culture of these individuals’ cells (see below). CD8\(^+\) T cells from strong responder A21 equally controlled CD4\(^+\) T cell superinfection by HIV-BaL and by autologous virus (Fig. 1D). In contrast, the weak CD8-mediated HIV suppression in subject A13 was not improved when his autologous virus was used to challenge his CD4\(^+\) T cells (0.01 vs. 0.16 log p24 decrease with HIV-BaL and the autologous virus, respectively) (Fig. 1D). CD8\(^+\) T cells from weak responder A19 showed a stronger capacity to inhibit infection by autologous viruses (0.33 vs. 1.76 log p24 decrease for HIV-BaL and autologous virus infection, respectively) (Fig. 1D), although the level of suppression did not reach that observed in strong responders. Interestingly, while most HICs were infected by subtype B viruses, subject A19 was infected by HIV-1 subtype A2 (Table I). Therefore, although the use of nonautologous viruses might lead to an underestimation of the HIV-suppressive activity of CD8\(^+\) T cells, it was unlikely to explain the differences observed between weak and strong responders.

**CD8\(^+\)-mediated HIV-suppressive capacity in HICs correlates with the frequency of IFN-γ-producing cells**

We examined whether the difference between strong and weak responder HICs was associated with a difference in the magnitude of HIV-specific CD8\(^+\) T cell responses. To quantify the HIV-specific CD8\(^+\) T cell response, we used the standard determination of the frequency of IFN-γ-secreting CD8\(^+\) T cells upon stimulation with HIV peptides.

![Figure 2](http://www.jimmunol.org/)

**FIGURE 2.** A, Frequencies of HIV-specific IFN-γ-secreting CD8\(^+\) T cells in strong responder HICs (log p24 decrease >2) (SR) and in weak responder HICs (log p24 decrease <2) (WR). An average of 36 ± 9 peptides were tested in each subject, depending on the results of HLA typing. Each symbol corresponds to the sum of SFCs/10\(^6\) PBMC obtained with individual peptides described as being restricted by HLA Ags. Horizontal lines are median values for each group. B, Correlation between the HIV-suppressive capacity of CD8\(^+\) T cells from HICs (log p24 decrease as shown in Fig. 1C) and their frequency of IFN-γ-producing CD8\(^+\) T cells upon HIV peptide stimulation. Each symbol represents one HIC. Vertical dashed line separates weak responder and strong responder HICs. C, Percentage of HIV-specific cells (based on HIV multimer and CD8 expression) from strong and weak responder HICs that expressed *ex vivo* HLA-DR and CD38, coexpressed CD27 and CD45RA, or proliferated (and lost CFSE labeling) after 5 days of peptide stimulation. Each symbol represents one specificity for one HIC. Horizontal lines are mean values for each group.
appropriate HLA-defined optimal HIV-1 Env, Gag, Pol, and Nef peptides in an ELISPOT assay. The numbers of IFN-γ-secreting cells were heterogeneous (Fig. 2A), in agreement with recent reports (20, 24). The highest frequencies of HIV-specific CD8+ T cells were observed in strong responders (8517 ± 4038 vs 1058 ± 903 SFCs/10^6 PBMC in weak responder HICs, p = 0.0014) (Fig. 2A). The frequency of HIV-specific CD8+ T cells in HICs was not significantly different, as a whole (6843 ± 4866 SFCs/10^6 PBMC), from that observed in chronically viremic patients (4616 ± 4148 SFCs/10^6 PBMC for 18 patients with >3 years of infection and plasma viral load >1000 RNA copies/ml, p = 0.20). The magnitude of the CD8+ T cell response in weak responder HICs was similar to that in HAART-treated patients (865 ± 1071 SFCs/10^6 PBMC for 11 patients with >2 years of treatment and plasma viral load <50 RNA copies/ml, p = 0.50; and Ref. 32).

Interestingly, we found a strong correlation between the frequency of IFN-γ-producing CD8+ T cells upon peptide stimulation and the HIV-suppressive capacity of unstimulated CD8+ T cells (Spearman 0.835, p < 0.00001) (Fig. 2B). This supports the possibility that the ex vivo anti-HIV activity of CD8+ T cells from HICs is driven by HIV-specific cells, in keeping with an MHC class I-mediated mechanism (21). This correlation further distinguished strong and weak responder HICs (Fig. 2B).

We explored whether differences could also be observed between strong and weak responder HICs at the phenotypical level of their HIV-specific CD8+ T cells. Due to the low frequency of these cells in weak responders, we could perform these analyses only in three of them. HIV-specific CD8+ T cells from strong responder HICs possessed a discordant activation phenotype with high expression of the activation marker HLA-DR associated with a low CD38 expression (Fig. 2C), in keeping with our previous study (21). In contrast, the expression of both activation markers was low in the cells from weak responders (Fig. 2C), a phenotype that is found in HAART subjects (21). HIV-specific CD8+ T cells from both strong and weak responders had high proliferative potential (Fig. 2C), which is a hallmark of a high-quality HIV-specific CD8+ T cell response in HICs (26). Interestingly, we found in weak responders an increase of a subpopulation of HIV-specific CD8+ T cells characterized by the coexpression of CD27 and CD45RA (Fig. 2C). We have recently reported that this subpopulation is characteristically abundant in HIV patients treated during acute primary HIV infection and may represent a stable quiescent long-term memory pool (33).

CD8-mediated HIV-suppressive capacity in HICs correlates strongly with the magnitude of Gag-specific CD8+ T cell responses

The response to Gag contributed most (average, 51.8%) to the total HIV-specific CD8+ T cell response (Fig. 3A). In strong responder HICs the contribution of the Gag response was 56.8% on average compared with 37.9% in weak responder HICs (p = 0.14). Responses to Nef peptides also contributed significantly to the overall CD8+ T cell response in HICs (average, 31.8% in strong responders and 31.2% in weak responders) (Fig. 3A). The contributions of Env and Pol responses were much smaller (7.9% and 8.7%, respectively, in the whole HIC population) (Fig. 3A). The contributions of the responses to the different HIV proteins were not different in HICs than in viremic patients (not shown), although a tendency was observed to a greater contribution of Gag responses in strong responder HICs than in viremics (average, 38%; p = 0.08). The magnitude of the Gag response was higher in HICs (3682 ± 2969 SFCs/10^6 PBMC) than in viremics (1703 ± 2061 SFCs/10^6 PBMC, p = 0.05). In contrast, Gag responses contributed less and Nef responses more to the total HIV-specific CD8+ T cell response in HAART-treated patients (14.3% and 60.6% of Gag and Nef responses) than in HICs (p = 0.014 and p = 0.025, respectively).

We then examined the influence of the specificity of HIC CD8+ T cells on the efficiency of HIV suppression. The correlation between the HIV-suppressive capacity of nonstimulated CD8+ T cells and the frequency of IFN-γ-producing CD8+ T cells upon peptide stimulation was strongest for Gag peptides (Spearman

![FIGURE 3](http://www.jimmunol.org/)
CD8-mediated HIV-suppressive capacity in strong responder HICs is broad

We have already reported a broad capacity of CD8$^+$ T cells from strong responder HICs to effectively control superinfection by different HIV-1 subtypes (21). Interestingly, CD8$^+$ T cells from strong responder HICs also partially suppressed infection of CD4$^+$ T cells by other human lentiviruses such as HIV-2, SIVmac, and SIVagm (Fig. 4C). At least some of the HIV-1 epitopes recognized by HIV-specific CD8$^+$ T cells from strong responders HICs seemed to strongly contribute to the HIV-suppressive capacity of CD8$^+$ T cells in all three strong responder HICs evaluated, in agreement with the correlations described above. In contrast, the contribution of Nef responses was more variable.
Weak responder HICs carry infectious replicative viruses

A recent report by Hatano et al. suggested that low level viral replication is ongoing in most HICs (22). We thus examined whether differences between strong responder and weak responder HICs might exist at a virological level. Ultrasensitive viral load tests were not available for this study. However, the long documented virological follow-up of the patients in the study, we had access to multiple RNA viral load determinations for all HICs (Table I). The length of the follow-up and the number of viral load determinations were similar for strong responder and weak responder HICs (p = 0.309 and p = 0.515, respectively; Table I). Interestingly, historical plasma viral load results showed that small blips of viral RNA were more frequently detected during follow-up among strong responder HICs than among HICs with weak CD8+ T cell responses, who appeared to control HIV infection more tightly (p = 0.016; Fig. 5A and Table I).

HIV-1 DNA level in blood cells, which is a stable parameter that gives an estimation of the HIV-1 reservoir size (34), was available for most HICs (Table I). Despite the differences in the frequency of viral RNA blips mentioned above, proviral DNA levels were very low in all the HICs, regardless of the strength of their CD8+ T cell responses (Fig. 5B). We then investigated whether autologous viral replication might be activated upon stimulation of CD4+ T cells from HICs. Surprisingly, replication-competent viruses were more readily detected in the supernatants of activated CD4+ T cells from weak responders than from strong responders (Fig. 5C). Moreover, autologous virus production upon CD4+ T cell stimulation correlated negatively with the HIV-suppressive capacity of CD8+ T cells (Spearman -0.635, p = 0.01). We obtained enough autologous viruses from weak responder HICs A13, A19, and A22 to test their infectivity. These viruses were able to spread and infect heterologous CD4+ T cells as efficiently as other laboratory-adapted strains and primary isolates (Fig. 5D). Their titers (6.1, 5.6, and 5.5 50% tissue culture-infective dose/ml for vA13, vA19, and vA22, respectively) were also similar (5.4 TCID50/ml for both BaL and NL4.3, and 6.1 TCID50/ml for v30007). Therefore, at least some HICs with weak CD8+ T cell responses carry viruses highly replicative in vitro. This is in agreement with recent reports showing that defective or attenuated viruses do not generally account for the control of viral replication in HICs (35–37).

Discussion

Here we show that the HIV-suppressive capacity of CD8+ T cells from HIV controllers is stable over time and is associated with the magnitude of HIV-specific CD8+ T cell responses, in particular to those directed against Gag. We also identify a group of HICs who carry infectious viruses and are able to durably control HIV infection despite a weak HIV-suppressive capacity of their CD8+ T cells.

Most of the HIC subjects in our study (14 of 19) had CD8+ T cells with marked and stable HIV-suppressive capacities (strong responder HICs, p24 log decrease >2) that we have never observed in viremic (21) or HAART-treated individuals. The protective HLA alleles B27 and/or B57 were present in all strong responder HICs. However, CD8+ T cells from a subgroup of HICs...
had only weak HIV-suppressive capacity. In agreement with recent reports (20, 24), the HICs we studied had heterogeneous levels of HIV-specific CD8\(^+\) T cells, as estimated by the frequency of IFN-γ-producing CD8\(^+\) T cells. The magnitude of the HIV-specific CD8\(^+\) T cell response correlated strongly with the capacity of CD8\(^+\) T cells from HICs to control HIV infection of autologous CD4\(^+\) T cells in vitro. Accordingly, the lowest frequencies of IFN-γ-producing CD8\(^+\) T cells were found in weak responder HICs.

Some underestimation of the CD8\(^+\) T cell response in HICs may come for the use of peptides derived from consensus sequences for ELISPOT determinations, or of a laboratory-adapted HIV strain for HIV-suppression analyses. However, CD8\(^+\) T cells from two weak responder HICs had limited suppressive capacity even when autologous viruses were used, which further supported a truly weak CD8\(^+\) T cell response in these individuals. We cannot exclude that control of viremia in weak responders may be due to robust HIV-specific CD8\(^+\) T cell responses in lymphoid tissues, and actually Ferre and colleagues have recently shown that HICs have polyfunctional HIV-specific T cell responses in rectal mucosa that were frequently stronger than in blood (38). However, the presence in this study of a few HICs with very weak responses both in the blood and in the rectal mucosa is interesting. Although a weak high quality CD8\(^+\) T cell response might be sufficient to control viremia in vivo, it seems unlikely to be the case in weak responder HICs. The absence of viral blips during the follow-up of weak responders and our finding that at least some of these HICs carry viruses that are highly infectious in vitro and readily detectable upon in vitro activation endorse the idea of a very tight and active host-restraint of HIV-1 infection. Our phenotypical analyses of the HIV-specific CD8\(^+\) T cells in weak responders showed an increased proportion of a CD27\(^-\)/CD45RA\(^-\) subset of cells, previously observed in patients treated during primary HIV infection, and that might represent a quiescent and stable memory pool able to proliferate and acquire effector capacities upon Ag stimulation (33). Although these cells may provide an effective response in the eventuality of viral replication, their increased proportion in weak responder HICs together with the low expression of HLA-DR suggest a long period without antigenic stimulation of the CD8\(^+\) T cell response.

Therefore, an alternative mechanism is probably responsible for controlling HIV-1 in these HICs. The lower antiviral activity of CD8\(^+\) T cells in weak responder HICs did not seem to be compensated for by other cell populations within PBMC (e.g., NK cells or γδ T cells), as illustrated by HIV-suppressive experiments where nonstimulated PBMC (depleted of CD4\(^+\) cells), used instead of CD8\(^+\) T cells, were also unable to control HIV superinfection of autologous CD4\(^+\) T cells (not shown). Interestingly, persistent lack of low-level detectable viremia in one HIC has been recently associated to low levels of HIV Abs and remarkably low levels of T cell activation (22). Further virologic studies (such as viral sequencing or determination of tissular viral replication) and the analysis of innate responses and regulatory T cells (39) might help to identify new mechanisms of control in HICs.

Unlike the cells from weak responder HICs, CD8\(^+\) T cells from strong responder HICs had a broad capacity to suppress superinfection of their own CD4\(^+\) T cells by a wide range of HIV-1 strains (21) and, at least partially, by other lentiviruses. This could be related to the presence of HIV-specific CD8\(^+\) T cells targeting epitopes located within highly conserved regions of the virus. Responses against Gag and Nef epitopes together accounted for the bulk of total CD8\(^+\) T cell responses in strong responder HICs, and no phenotypic differences were observed between Gag-specific and Nef-specific CD8\(^+\) T cell responses in these individuals (21). Interestingly, we observed a strong correlation between the HIV-suppressive capacity of CD8\(^+\) T cells in strong responder HICs and the number of Gag-specific CD8\(^+\) T cell responses. Moreover, the analysis of the relative HIV-suppressive capacity of the Gag response in three strong responder HICs showed that, for all three HICs, Gag-specific CD8\(^+\) T cells possess the strongest antiviral capacities. Thus, Gag responses appear to be strongly involved in the antiviral potency of CD8\(^+\) T cells. This is in agreement with a report showing evidence of CD8\(^+\) T cell selective pressure on gag in HICs (40). Increasing evidence suggests that Gag-specific CD8\(^+\) and CD4\(^+\) T cell responses are associated with better control of HIV viremia (7–9, 20, 41). Gag epitopes are presented on the surface of infected CD4\(^+\) T cells early after viral entry, before DNA integration and viral protein synthesis (42), and this might allow Gag-specific CD8\(^+\) T cells to recognize and eliminate infected cells before the infection is properly established and before Nef-mediated down-regulation of MHC class I molecules occurs (43). Other factors such as functional avidity (41, 44) or lytic granule loading (45) might contribute to an enhanced HIV-suppressive capacity of Gag-specific CD8\(^+\) T cells.

No correlation was found between HIV-suppressive capacity of CD8\(^+\) T cells in strong responder HICs with Nef-specific CD8\(^+\) T cell responses. However, this observation does not preclude a contribution of responses targeting Nef (or other viral proteins) to the HIV-suppressive capacity of CD8\(^+\) T cells. Actually, our experiments of selective depletion of HIV-specific cell fractions showed variable capacities (from strong to none) of Nef-specific CD8\(^+\) T cells from HICs to suppress HIV infection, perhaps depending on the frequency of the Nef responses that were targeting epitopes restricted by HLA-B57. Along these lines, escaping mutations are also found in Nef epitopes in HICs, although less frequently than in Gag epitopes (46).

Escaping mutations in epitopes located within structurally important regions of the virus could limit the capacity of the virus to mutate to escape immune pressure, as variations in these regions have a fitness cost (13, 47). Although we did not directly address this issue, the difficulties to detect HIV-1 replication in the culture supernatants of activated CD4\(^+\) T cells from strong responder HICs might reflect the impact of the pressure exerted by CD8\(^+\) T cell responses on viral fitness. Nevertheless, we cannot exclude that, given the extraordinary antiviral potency of CD8\(^+\) T cells from strong responder HICs, the few remaining CD8\(^+\) T cell responses in these experiments were enough to efficiently suppress autologous virus replication.

Several important questions await answers; that is, mainly whether the potent CD8\(^+\) T cell response observed in most HICs precedes or follows initial viremic control, and how such a potent CD8\(^+\) T cell response is maintained. The association presented here between blips in plasma viral RNA and stronger CD8\(^+\) T cell responses in HICs must be considered with care because of the limited number of weak responder HICs, but it is tempting to speculate that CD8\(^+\) T cell control of HIV might involve a feedback mechanism whereby occasional blips (or ongoing low-level viral replication) are needed to boost the antiviral response. The increased telomerase activity in these cells would further ensure their persistence (28). Two scenarios can be envisaged: 1) if viremia is controlled by a common mechanism in weak and strong responder HICs, the presence of the protective HLA B27 and B57 alleles may help to sustain control over time, in the eventuality of viral escape, through the establishment of a robust CD8\(^+\) T cell response; 2) different mechanisms are responsible for initial control of HIV infection in weak and strong responder HICs. Detailed longitudinal studies of HICs will be necessary to answer these questions.
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Disclosures

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