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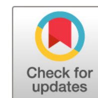
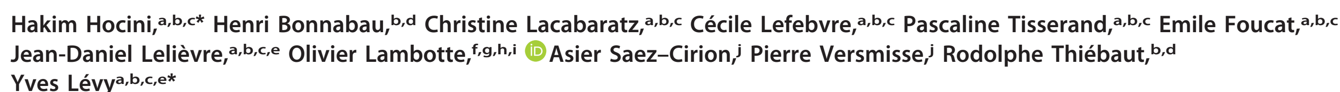


HIV Controllers Have Low Inflammation Associated with a Strong HIV-Specific Immune Response in Blood



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ABSTRACT HIV controllers (HIC) maintain control of HIV replication without combined antiretroviral treatment (cART). The mechanisms leading to virus control are not fully known. We used gene expression and cellular analyses to compare HIC and HIV-1-infected individuals under cART. In the blood, HIC are characterized by a low inflammation, a downmodulation of natural killer inhibitory cell signaling, and an upregulation of T cell activation gene expression. This balance that persists after stimulation of cells with HIV antigens was consistent with functional analyses showing a bias toward a Th1 and cytotoxic T cell response and a lower production of inflammatory cytokines. Taking advantage of the characterization of HIC based upon their $CD8^+$ T lymphocyte capacity to suppress HIV-infection, we show here that unsupervised analysis of differentially expressed genes fits clearly with this cytotoxic activity, allowing the characterization of a specific signature of HIC. These results reveal significant features of HIC making the bridge between cellular function, gene signatures, and the regulation of inflammation and killing capacity of HIV-specific $CD8^+$ T cells. Moreover, these genetic profiles are consistent through analyses performed from blood to peripheral blood mononuclear cells and T cells. HIC maintain strong HIV-specific immune responses with low levels of inflammation. Our findings may pave the way for new immunotherapeutic approaches leading to strong HIV-1-specific immune responses while minimizing inflammation.

IMPORTANCE A small minority of HIV-infected patients, called HIV controllers (HIC), maintains spontaneous control of HIV replication. It is therefore important to identify mechanisms that contribute to the control of HIV replication that may have implications for vaccine design. We observed a low inflammation, a downmodulation of natural killer inhibitory cell signaling, and an upregulation of T-cell activation gene expression in the blood of HIC compared to patients under combined antiretroviral treatment. This profile persists following *in vitro* stimulation of peripheral blood mononuclear cells with HIV antigens, and was consistent with functional analyses showing a Th1 and cytotoxic T cell response and a lower production of inflammatory cytokines. These results reveal significant features of HIC that maintain strong HIV-specific immune responses with low levels of inflammation. These findings de-

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fine the immune status of HIC that is probably associated with the control of viral load.

KEYWORDS HIV controllers, HIV infection, immune mechanisms, immune response

If left untreated, HIV-1 infection is characterized by a detectable HIV replication and a rapid decline in $CD4^+$ T lymphocytes leading to AIDS, whereas a small minority of patients, called HIV controllers (HIC), maintains spontaneous control of HIV replication (1–3). Although this population is heterogeneous and several mechanisms leading to the control of HIV replication contribute to this outcome (4, 5), an efficient HIV-specific $CD8^+$ T cell response appears to be a key factors associated with the control of viremia. HIC maintain polyfunctional $CD8^+$ T cell responses to HIV-1 antigens (6, 7), in particular to gag polypeptide (8). A population of HIC exhibiting strong functional HIV-specific cytotoxic $CD8^+$ T cell responses (2) has been characterized (9). Indeed, primary $CD8^+$ T cells from many HIC are able to suppress HIV-1 replication *ex vivo* by efficient granzyme B- and perforin-mediated killing of infected T cells (10).

In previous reports (9, 11), we defined two subgroups of HIC based on the capacity of their $CD8^+$ T cells to suppress HIV-1 infection *ex vivo* in autologous $CD4^+$ T lymphocytes (12). Strong-responder HIC (SRHIC) exhibit a higher $CD8^+$ T cell HIV-suppressive capacity than weak-responder HIC (WRHIC). It was also observed that WRHIC maintain a large pool of HIV Gag-specific central memory T cells that are highly functional and readily expandable upon antigen stimulation, able to reach functions and a high frequency similar to those observed in SRHIC (13). A negative correlation between expandable Gag-specific memory T cell responses and residual viremia suggests that these cells actively contribute to the sustained suppression of virus replication (14).

In order to identify mechanisms that may contribute to the spontaneous control of HIV replication in HIC, we hypothesized that comparison of blood gene expression profiles of HIC and chronically HIV-infected patients, with high $CD4^+$ T cell counts and suppressed plasma HIV loads while on combined antiretroviral treatment (cART), might help to identify features of spontaneous HIV control. In a second approach, cellular and genetic analyses of the peripheral blood mononuclear cells (PBMC) of these patients stimulated *in vitro* with HIV antigens were performed. Finally, in order to further characterize the SRHIC and WRHIC, we compared gene profiles of purified $CD4^+$ and $CD8^+$ T lymphocytes. Globally, our results identified key profiles of immune control of viral replication delineating implications for the design of strategies aimed to a sustained remission of HIV infection.

RESULTS

Characteristics of the study population. The blood samples of the cohort were drawn from 53 HIC subjects and 27 cART-treated patients. Clinical characteristics of the two groups are shown in Table 1. No statistically differences were observed between the two groups in terms of age (median of 47 versus 52 years old), viral load (1.6 versus 1.3 RNA $\log_{10}$ copies/ml), and $CD4^+$ T (689 versus 588 cells/mm^3) and $CD8^+$ T (829 versus 725 cells/mm^3) cell counts. No statistically differences were also observed for these parameters between the 10 SRHIC and 9 WRHIC subjects used for purification of CD4 and CD8 T cells and between HIC and cART used for PBMC purification.

HIC are characterized by an increase in T cell activation and a downmodulation of inflammatory genes in the blood. Gene expression profile analysis of whole blood of HIC ($n = 53$) and cART patients ($n = 27$) showed that 1,244 genes were differentially expressed. Globally, these genes belong to pathways involved in innate immunity and natural killer (NK) cell signaling, T cell activation and inflammation. HIC were clearly characterized by a downmodulation of genes related to inflammation response with a downregulation of Toll-like receptors (TLRs) and TREM1 pathways (*TLR1* [−1.73], *TLR4* [−1.91], *TLR6* [−1.87], *TLR8* [−2.61], *CD14* [−1.66], *TREM1* [−2.12], and *TYROBP* [−1.62]) and of many proinflammatory genes, including neutrophil chemotactic factor *IL-8/*

TABLE 1 Characteristics of HIC and cART subjects

Characteristic	HIC	cART patients
No. of subjects	53	27
Age, yr (Q1/Q3)	47 (20/79)	52 (40/64)
Gender (no. female; no. male)	24; 29	12; 15
HIV-1 plasma viral load (RNA copies/ml)		
Mean (SD)	1.6 (0.46)	1.3 (0.16)
Median (Q1/Q3)	1.4 (1.3/1.9)	1.3 (1.3/1.3)
$CD4^+$ lymphocytes		
No. of subjects	53	27
Count (cells/mm^3)		
Mean (SD)	713 (249)	606 (186)
Median (Q1/Q3)	689 (502/859)	588 (498/698)
$CD8^+$ lymphocytes		
No. of subjects	50	27
Count (cells/mm^3)		
Mean (SD)	829 (398)	725 (330)
Median (Q1/Q3)	794 (593/920)	681 (526/852)

CXCL8 (−8.14) and its receptors *CXCR1* (−3.12) and *CXCR2* (−4.09) (Fig. 1A). More precise analysis also revealed a downmodulation in HIC of receptors for the Fc portion of immunoglobulin (*FCGR3A/FCGR3B* [−7.65], *FCER1G* [−1.94]), including the CD32A gene (*FCGR2A* [−2.77]), as well as killer cell immunoglobulin-like receptors (*KIR2DL1/KIR2DL3* [−1.85], *KIR3DL1* [−1.82], *KIR2DL4* [−1.7], and *KIR2DL5A* [−1.25]) and killer cell lectin-like receptors (*KLRD1* [−1.76], *KLRC3* [−1.96], and *KLRC2* [−1.69]) (Fig. 1A). This result contrasts with an upregulation in HIC of the expression of Src family kinases, *FYN*

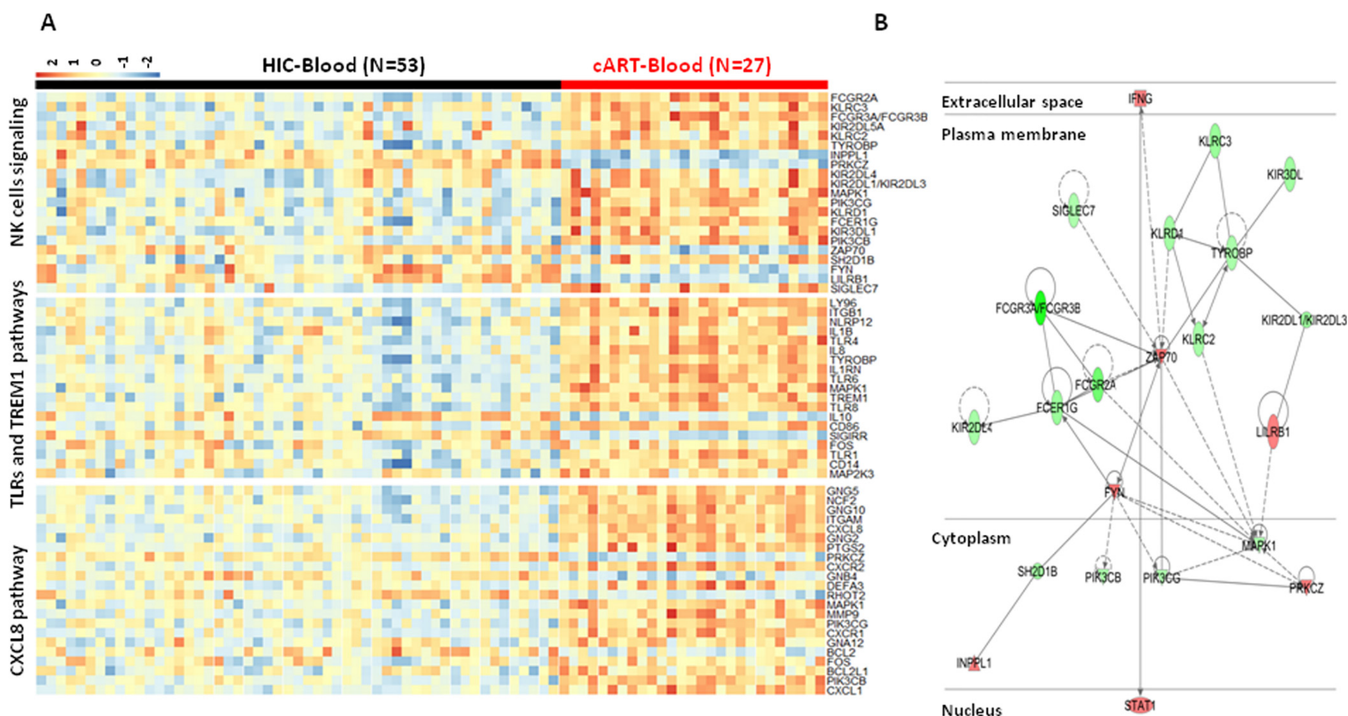

FIG 1 Gene expression in HIC and cART patients in whole blood. (A) Heatmap of genes belonging to the main pathways associated with differentially expressed genes in whole blood of HIC and cART patients, including NK cells, TLRs, and TREM1 and CXCL8 pathways. (B) Relationships between genes differentially expressed in whole blood of HIC compared to cART patients. Red symbols are overexpressed genes in HIC compared to cART patients; green symbols are underexpressed genes. Solid lines represent direct links between genes, and dashed lines represent indirect links (with no more than one gene between the two genes).

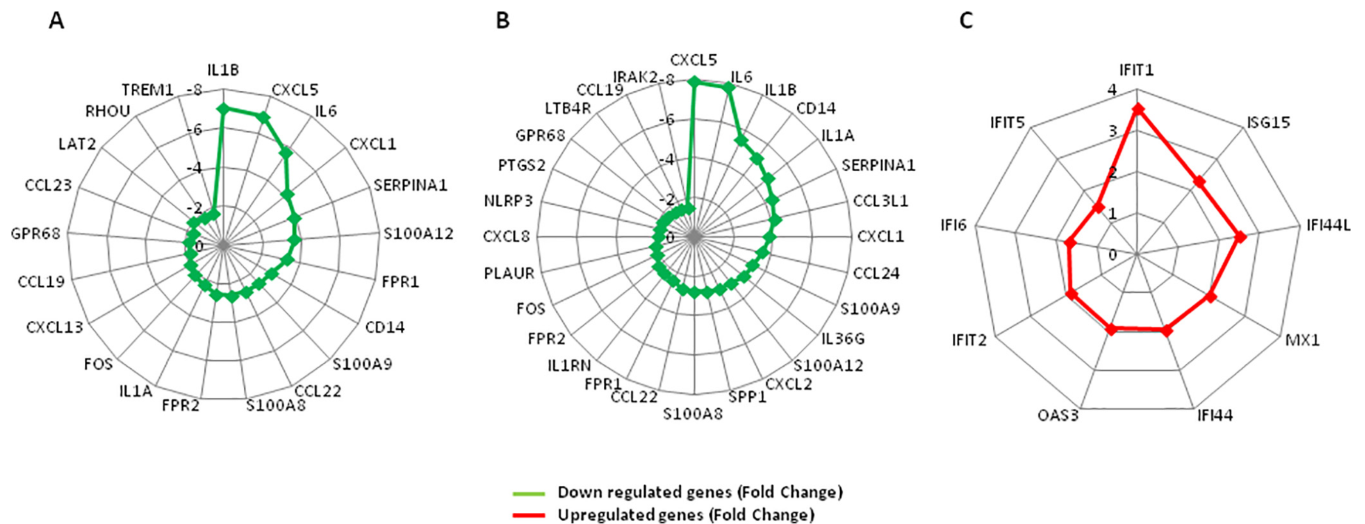

FIG 2 Gene expression in HIV peptides unstimulated and stimulated PBMC of HIC and cART patients. (A) Main differentially expressed genes between HIC and cART patients associated with inflammation before HIV peptide stimulation. (B and C) Differentially expressed genes between HIC and cART patients associated with inflammation and IFN signaling after HIV peptide stimulation of PBMC.

(+1.66) and *ZAP70* tyrosine kinase (+1.73), *IFNG* (+1.51), and *STAT1* (+1.54) (Fig. 1B) genes. Interestingly, the low inflammatory profile in HIC is consistent with the downmodulation of inflammation regulatory pathways, mitogen-activated protein kinase 1 (*MAPK1* [−1.59]), and phosphatidylinositol 3-kinase (PI3-kinase; *PIK3CG* [−1.52] and *PIK3CB* [−*1.61*]) (Fig. 1B), a critical regulatory factor that connects immune stimulation and suppression during inflammation (15, 16). Globally, as illustrated in Fig. 1B, analysis revealed significant direct interactions between these pathways linking the downmodulation of *PIK3CG* with an increase in T cell activation (*ZAP70/FYN*) and a decrease in the innate cell inhibitory signaling of NK cells (*KIRs*).

We have also looked for immunometabolism pathways that play important role in the modulation of the immune system. In whole blood, we have observed an enrichment of the gluconeogenesis and lipid metabolism pathways. In that respect, we observed a downregulation in HIC compared to cART of *ALDOA* (aldolase, fructose-bisphosphate A [−1.57]), *BPGM* (bisphosphoglycerate mutase [−2.31]), *ME2* (malic enzyme 2 [−1.61]), *PGAM1* (phosphoglycerate mutase 1 [−1.54]), and *PGAM4* (phosphoglycerate mutase 4 [−1.51]) and an upregulation of *ENO3* (enolase 3 [+1.64]). In lipid metabolism, there was a downmodulation of *PTGS2* (prostaglandin-endoperoxide synthase 2 [−2.88]), *CD36* (−2.24), *ACSL1* and *ACSL4* (acyl coenzyme A synthetase long-chain family members [−2.97 and −1.56]), *S1PR1* and *S1PR3* (sphingosine-1-phosphate receptors [−1.62 and −1.70]), *PCTP* (phosphatidylcholine transfer protein [−2.13]), and *PTGS2* (prostaglandin-endoperoxide synthase 2 [−2.88]). In contrast, *PTGR2* (prostaglandin reductase 2 [+2.15]), *PLA2G2D* (phospholipase A2 group IID [+2.18]), and *SREBF1* (sterol regulatory element binding transcription factor 1 [+1.55]) were upregulated in HIC compared to cART-treated subjects. However, the modulated genes did not allow us to predict an activation or inhibition of these pathways in HIC compared to cART-treated subjects.

HIC cellular responses to HIV peptides are associated with a low inflammatory gene expression associated with Th1 and cytotoxic profiles. We analyzed the differences in gene expression of PBMC isolated from HIC ($n = 25$) and cART patients ($n = 15$) before and after *in vitro* stimulation with pools of HIV peptides.

Gene expression analysis before HIV peptides stimulation revealed that 113 genes were differentially expressed. Ingenuity Pathway Analysis software showed that these genes are significantly involved in inflammation with a downregulation of many genes, such as *IL1A* (−2.28) and *IL1B* (−7.02), *IL-6* (−5.71), *CXCL5* (−6.89), *CXCL13* (−1.97), *CCL23* (−1.68), *CXCL1* (−4.19), *TREM1* (−1.66), and *CD14* (−2.86) (Fig. 2A). Some of

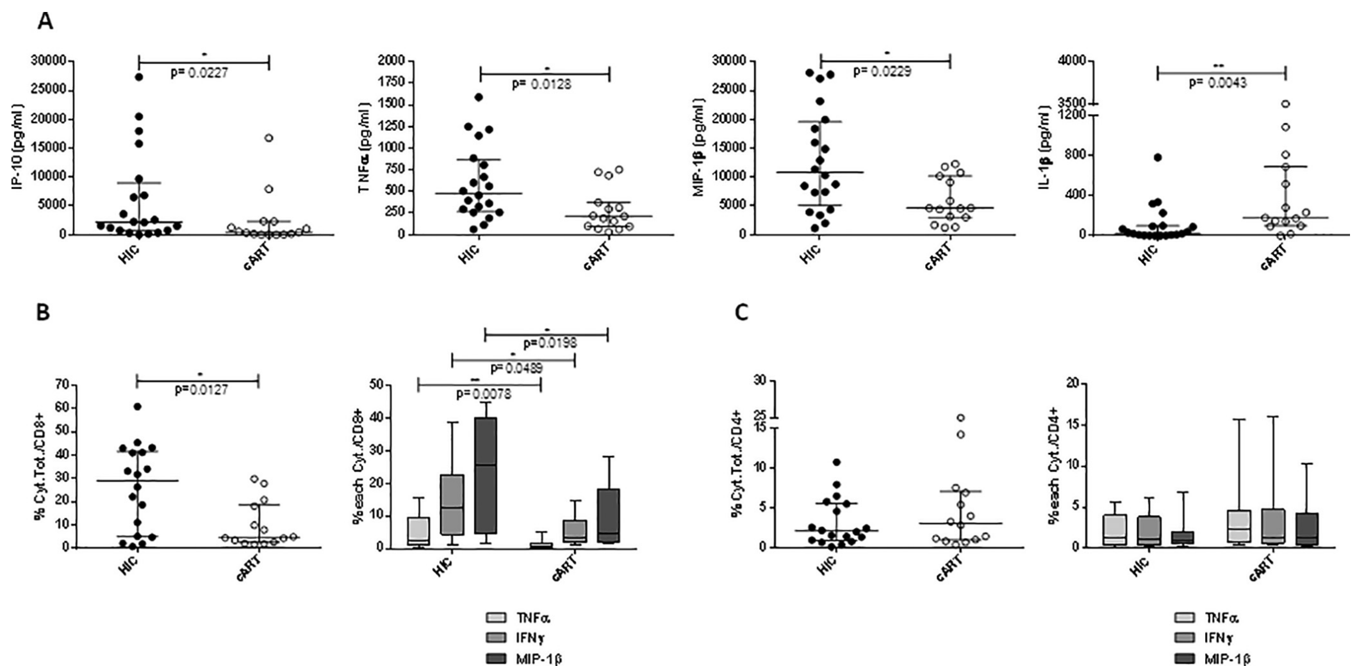

FIG 3 Cytokine profiles of PBMC from HIC and cART patients stimulated *in vitro* with HIV peptides covering Gag, Pol, and Nef antigens. (A) Cytokine measurements (pg/ml) in supernatants of stimulated PBMC from HIC ($n = 20$) and cART patients ($n = 15$). Cytokine secretion was measured in supernatants after HIV peptide stimulation of PBMC using Bio-Plex 200 system (Bio-Rad) at day 2 of stimulation for IP-10 and IL-1β and at day 9 after a restimulation for 24 h for TNF-α and MIP-1β. (B and C) CD8 and CD4 T-cell-producing cytokines after PBMC stimulation with HIV peptides for 9 days, as measured by ICS. (B) Frequency of CD8 T cells producing TNF-α, IFN-α, and MIP-1β (the sum of the cytokines or individual cytokines) in 18 HIC and 14 cART patients. (C) Frequency of CD4 T cells producing TNF-α, IFN-γ, and MIP-1β (the sum of the cytokines or individual cytokines) in 18 HIC and 14 c-ART patients. Horizontal lines represent the medians $\pm$ the interquartile ranges (IQR), and a Mann-Whitney test was applied.

these genes are also related to granulocyte adhesion and diapedesis (*IL1A*, *IL1B*, *CXCL5*, *CXCL1*, *FPR1*, *FPR2*, *CCL22*, *CXCL13*, *CCL19*, and *CCL23*) and to interleukin-6 (IL-6), HMGB, and TREM1 signaling (*IL1B*, *IL-6*, *CD14*, *IL1A*, *FOS*, *LAT2*, *RHOU*, and *TREM1*). We also observed a downregulation of genes involved in the iron homeostasis pathway, such as *HBA1/HBA2* (−12.93), *HBB* (−12.3), *HBG1* (−8.14), *HBG2* (−7.58), *IL-6* (−5.71), *ALAS2* (−4.52), *SLC11A1* (−2.24), and *SLC25A37* (−1.88). Likewise, gene expression analysis of HIV peptide-stimulated PBMC between HIC and cART revealed that 144 annotated genes were differentially expressed. Pathway analyses showed, as for unstimulated cells, downregulations of genes belonging to the inflammatory immune response, including *CD14* (−5.12), *CXCL8* (−1.84), *TREM1* (−1.71), and *IL-6* (−7.78), as well as *CXCL5* (−7.87), *IL1B* (−5.45), *IL1A* (−4.74), *CCL3L1* (−4.17), *CXCL1* (−3.82), and *CCL24* (−3.55) (Fig. 2B). We further observed a significant upregulation of genes related to the interferon pathway, such as *IFIT1* (+3.54), *IFI44L* (+2.50), *IFI44* (+1.94), *MX1* (+2.02), and *OAS3* (+1.91) (Fig. 2C).

These genetic characteristics were found to be consistent with the profile of cytokine production of *in vitro*-stimulated PBMC from HIC (20 samples) and cART patients (15 samples), as shown in Fig. 3. We observed a lower production of IL-1β and a higher production of IP-10, tumor necrosis factor alpha (TNF-α) and macrophage inflammatory protein 1β (MIP-1β) in HIC compared to cART-treated subjects, as measured by Luminex (Fig. 3A). This result was confirmed by intracellular cytokine staining (ICS) analysis after PBMC stimulation with HIV peptides showing a higher frequency of CD8$^+$ T cell producing TNF-α, MIP-1β, and gamma interferon (IFN-γ) in HIC patients compared to cART ($P = 0.0127$, Mann-Whitney test) (Fig. 3B). In contrast, no difference was observed in the profile of cytokine production for CD4 T cells from HIC and cART-treated subjects (Fig. 3C).

Genetic and functional analyses of CD8$^+$ T cells from SRHIC and WRHIC reveal specific signatures. We analyzed cytokine patterns of *in vitro* stimulated PBMC and

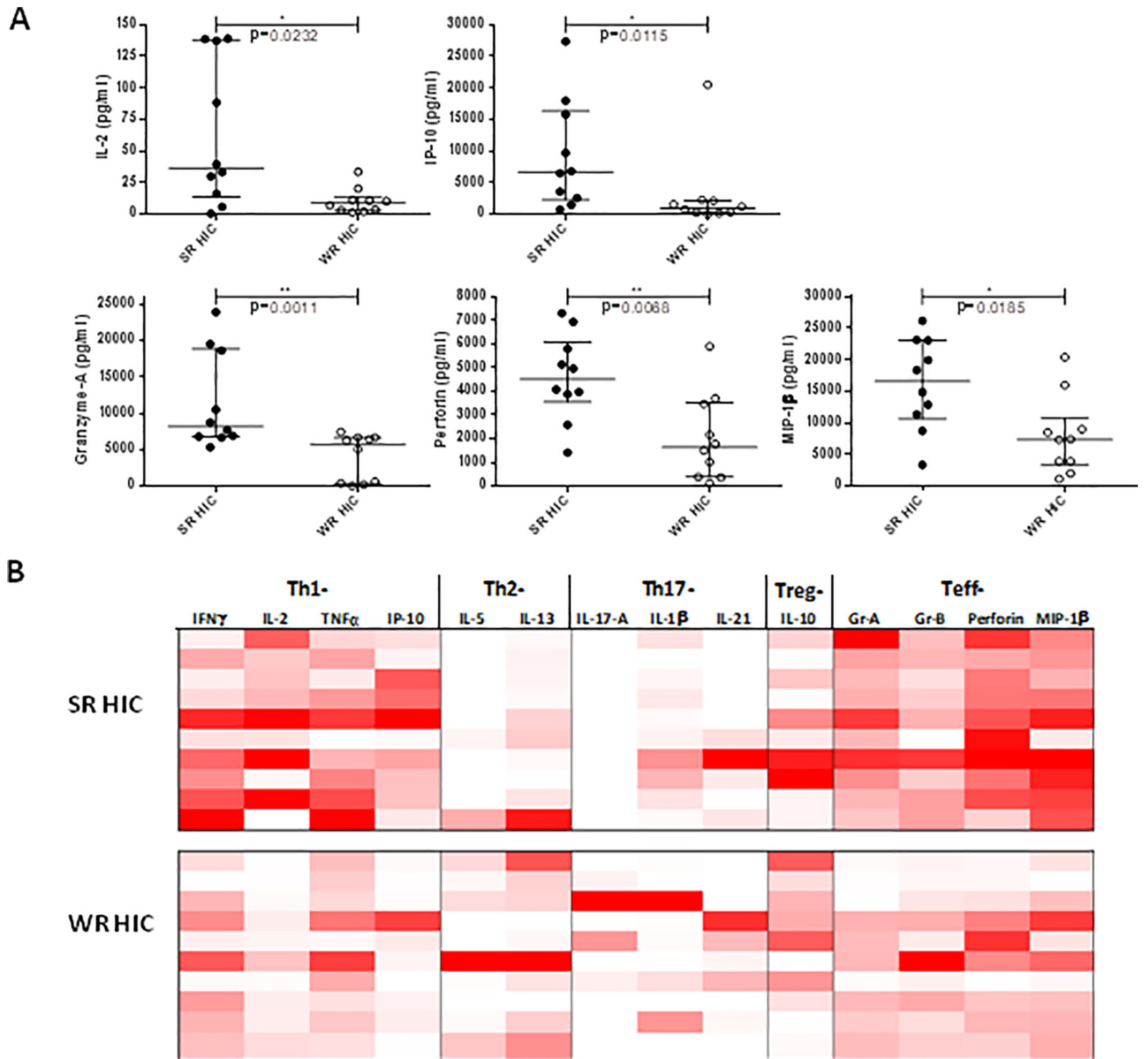

FIG 4 Cytokine profiles of PBMC from strong (SRHIC) and weak (WRHIC) HIC, stimulated *in vitro* with HIV peptides covering Gag, Pol, and Nef antigens. (A) Cytokine measurements (pg/ml) in supernatants of stimulated PBMC from 10 SRHIC and 10 WRHIC. Horizontal lines represent medians ± the IQR, and a Mann-Whitney test was used to compare the cytokine secretion among groups of patients. (B) Heatmap of 14 cytokine profiles of SRHIC and WRHIC. Cytokine secretion was measured in supernatants after HIV peptide stimulation of PBMC using Bio-Plex 200 system (Bio-Rad) at day 2 of stimulation for IL-2, IL-1β, and IP-10 or at day 9 after a restimulation for 24 h for all other cytokines. The white color indicates a very low cytokine concentration (or no detection), and the dark red color indicates a high cytokine concentration.

gene expression profiles of purified CD4 and CD8$^+$ lymphocytes from SRHIC and WRHIC. PBMC stimulation with HIV peptides led to a significant higher production of IL-2, IP-10, granzyme A, perforin, and MIP-1β in SRHIC compared to WRHIC (Fig. 4A), which is consistent with a stronger Th1- and T effector-cytokines response in SRHIC subjects (Fig. 4B). Phenotypic analyses in an ICS assay confirmed a higher frequency of CD8$^+$ T cells producing cytokines in SRHIC compared to the WRHIC group ($P = 0.031$, Mann-Whitney test), especially MIP-1β ($P = 0.024$) (Fig. 5A). CD8$^+$ T cells from SRHIC and WRHIC were highly polyfunctional (55 to 60% of cells exhibit two or three cytokines) in both groups. Although CD4$^+$ T cells from both SRHIC and WRHIC patients

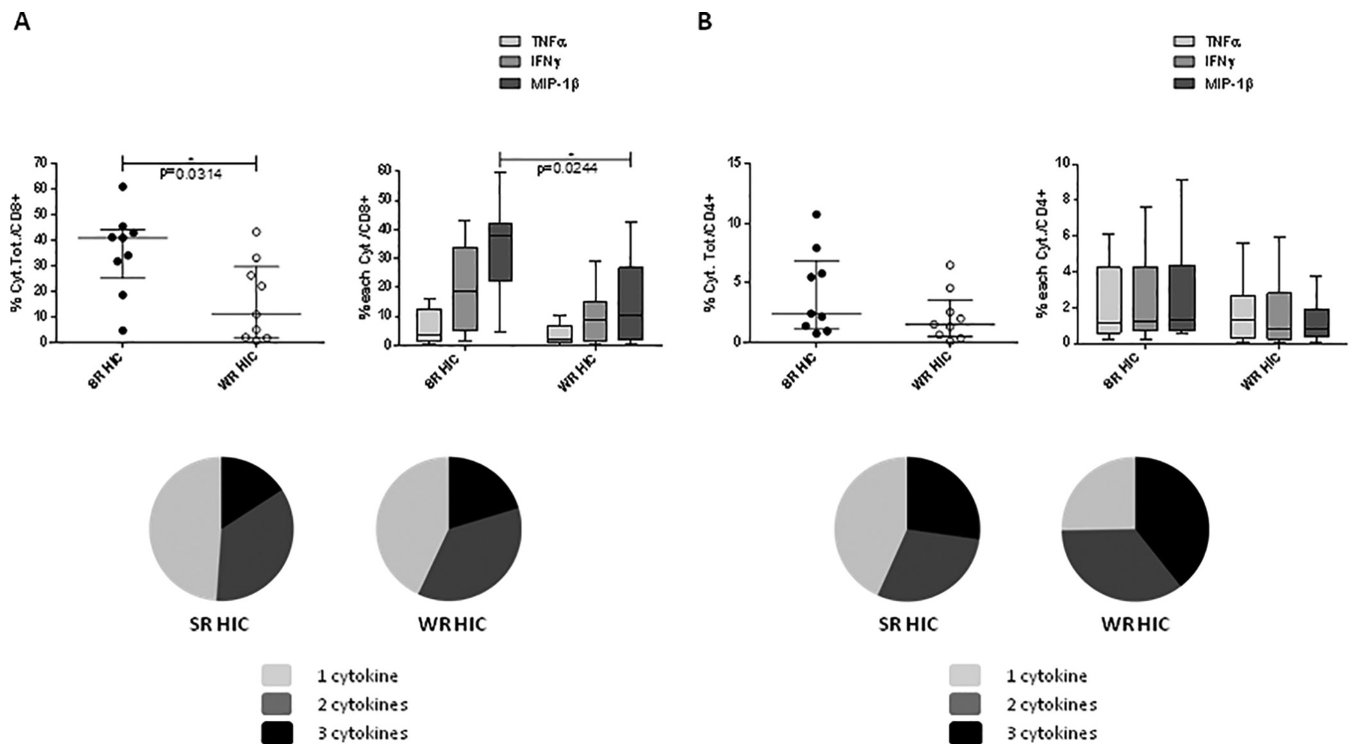

FIG 5 CD4 and CD8 T-cell-producing cytokines after PBMC stimulation with HIV peptides for 9 days, as measured by an ICS assay. (A) Frequency of CD8 T cells producing TNF-α, IFN-γ, and MIP-1β (the sum of the cytokines or individual cytokines) in 9 SRHIC and 9 WRHIC. (B) Frequency of CD4 T cells producing TNF-α, IFN-γ, and MIP-1β (the sum of the cytokines or individual cytokines) in 9 SRHIC and 9 WRHIC. Horizontal lines represent the medians ± the IQR, and a Mann-Whitney test was used. Pie charts represent the cell polyfunctionality, i.e., the relative proportions of CD8 and CD4 T cells producing one (gray), two (dark gray), or three (black) cytokines.

were highly polyfunctional (60 to 75% of the cells exhibit two or three cytokines), no differences were observed between groups in terms of cytokine production following HIV peptide stimulation (Fig. 5B).

We then compared gene expression profiles of *ex vivo* CD8$^+$ and CD4$^+$ T lymphocytes purified from SRHIC and WRHIC. In contrast to CD4$^+$ T lymphocytes (Fig. 6A), unsupervised hierarchical clustering analysis of CD8$^+$ T lymphocytes showed a perfect clustering of SRHIC and WRHIC groups (Fig. 6B). We found 804 annotated differentially expressed genes between SRHIC and WRHIC CD8 cells. Analysis of gene expression profiles of CD8$^+$ T lymphocytes showed an upregulation in SRHIC of genes involved in the IFN-γ pathway (Fig. 7), whereas proinflammatory genes such as *CXCL8* (−3.53), *IL1B* (−2.28), *IRAK3* (−1.61), *TYROBP* (−3.13), and *FCER1G* (−3.37) were downregulated. CD8$^+$ T lymphocytes from SRHIC exhibited also a significant upregulation of *CX3CR1* (+2.21) gene expression, a marker of CD8 effector memory cells (17).

Of the 804 genes differentially expressed between CD8$^+$ T cells from SRHIC and WRHIC, 133 were also part of those identified in blood gene expression differences between HIC and cART-treated subjects (Fig. 8A). These genes are mainly associated with a downmodulation of inflammation. Among the 671 genes differentially expressed specifically between CD8$^+$ T cells from WRHIC and SRHIC (excluding the 133 genes differentiating blood gene expression of HIC from cART-treated patients), four main functions were identified: three were predicted as "activated" (T cell response, cytotoxicity of leukocytes, and killing natural killer cells), and one was predicted as "inhibited" (activation of leukocytes). The downregulation of genes such as *NFKB1* was consistent with the decrease in leukocyte activation and the increase in leukocyte toxicity (Fig. 8B).

These data reveal significant features of HIC making the bridge between HIV-specific cellular function, i.e., polyfunctionality, low proinflammatory responses, cytotoxic ac-

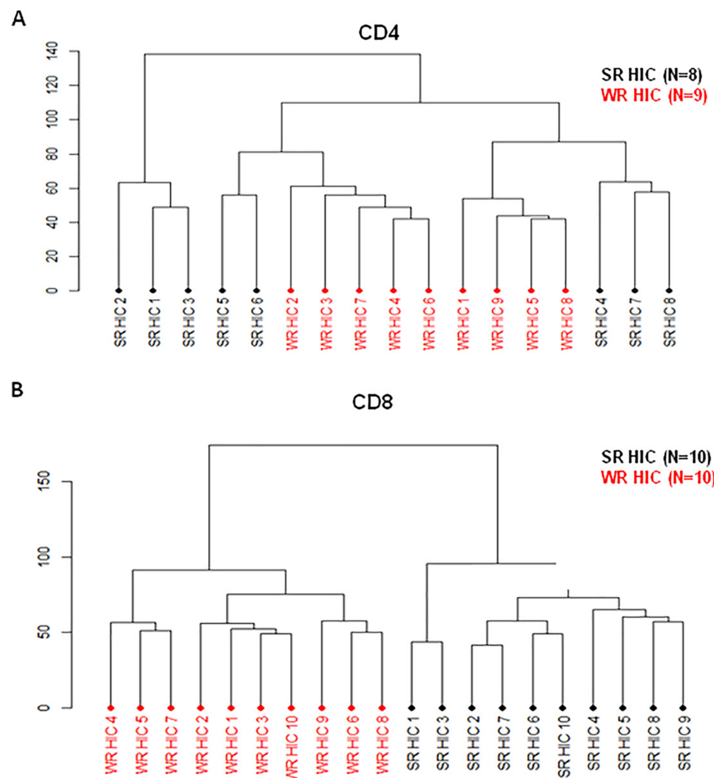

FIG 6 Unsupervised hierarchical clustering of differentially expressed genes between SRHIC and WRHIC subjects in purified T cell populations. (A) Unsupervised hierarchical clustering of $CD4^+$ T lymphocytes samples between 8 SRHIC and 9 WRHIC subjects. (B) Unsupervised clustering of $CD8^+$ T lymphocytes samples between 10 SRHIC and 10 WRHIC subjects.

tivity, and gene signatures. Interestingly enough, these genetic profiles are consistent through the analyses of *ex vivo* whole blood and PBMC to the analyses performed at the cellular population levels.

DISCUSSION

We report here results from extensive functional and gene expression analyses performed in whole blood and at the cellular level through PBMC and purified CD4 and CD8 T cells in a cohort of HIC. Globally, these analyses performed through the different compartments were consistent. They show that HIC individuals, compared to chronically HIV-1-infected individuals receiving cART, have a low inflammatory background that contrasts with activation of the adaptive immune response pathways. Interestingly, this balance persists following *in vitro* stimulation of cells with HIV antigens. This genetic profile was also consistent with functional analyses, as assessed by the production and cellular expression of cytokines. Finally, taking advantage of the characterization of HIC based upon their *in vitro* $CD8^+$ T lymphocyte capacity of killing HIV-infected cells, we show clearly that unsupervised genetic analysis of differentially expressed genes fits clearly with this cytotoxic activity. Here again, we found a balance between low activation and the commitment of genes associated with cytotoxicity and T cell response.

Although cART has significantly improved the prognosis of HIV-infected individuals, they remain at increased risk of morbidity and mortality (18, 19). These clinical events are supposed to be related to residual immune activation and inflammation in cART-treated patients. The immune activation is also associated with the poor HIV-specific response in chronically infected patients (20). Several studies have shown that HIC exhibit cellular and serological markers of immune activation and inflammation despite a spontaneous control of HIV replication (21–24). However, no evidence of persistent

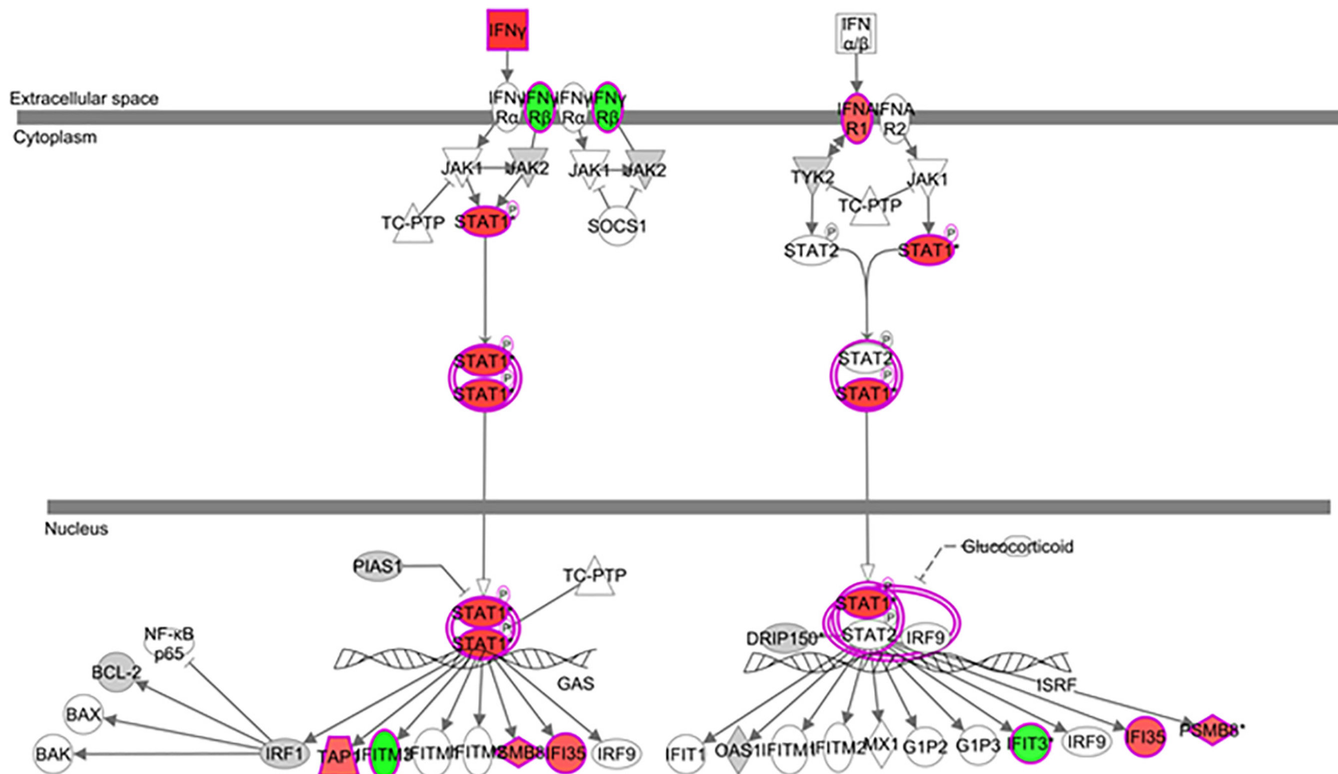

FIG 7 IFN-γ pathway associated with differentially expressed genes between SRHIC and WRHIC purified CD8 T cells. Overexpressed genes with an FC of ≥1.5 are represented in red, underexpressed genes are represented in green, and genes with FCs of <1.5 and >1.2 are represented in gray.

inflammation was observed when HIC were defined using stringent criteria in relation to the cutoff level of viremia (≤50 copies/ml) and a minimum follow-up time of >5 years, compared to HIV-uninfected subjects (25). We found here that, compared to cART patients, the level of inflammatory gene expression remains still dramatically reduced in HIC with a significant downregulation of TLRs, TRIM1, and CXCL8/IL-8. This result extends several observations showing that HIC have significantly lower levels of IL-8 mRNA when PBMC were exposed to exogenous HIV-1 compared to HIV progressors, whether cART treated or not, and HIV-uninfected controls (26). Also observed was a higher expression of CXCL8 in untreated HIV-1-infected progressors and cART nonresponders compared to long-term nonprogressors and cART responders, respectively. Furthermore, a negative correlation of plasma levels of CXCL8 with CD4 counts was found in HIV-1-infected cART-naive subjects, whereas the CXCL8 levels positively correlated with viral load in the cART-treated children (27). These observations suggest a strong link between CXCL8 through its proinflammatory action to viral replication and disease progression. On the other hand, El-Far et al. (28) underlined the role of the proinflammatory IL-32 cytokine in the failure of virus replication control in HIC. We did not find any differences between HIC and cART patients in the expression of IL-32 gene in our study, where there was no failure to control viral replication, neither in HIC nor in cART patients. In addition to the downregulation of inflammatory genes, HIC downregulated many genes belonging to the natural killer cell signaling pathway, such as receptors for the Fc portion of immunoglobulin, inhibitory killer cell immunoglobulin-like receptors, and killer cell lectin-like receptors. Interestingly, studies on HIV slow progressors linked the protective effect of NK cells with certain killer immunoglobulin-like receptors and their ligands the human leukocyte antigen-class I molecules (HLA) on target cells (29, 30). The responsiveness of NK cells varies depending on the number of inhibitory receptors (iKIR) expressed, in particular KIR2DL1/KIR2DL3 (29, 31, 32). Interestingly, expansion of the

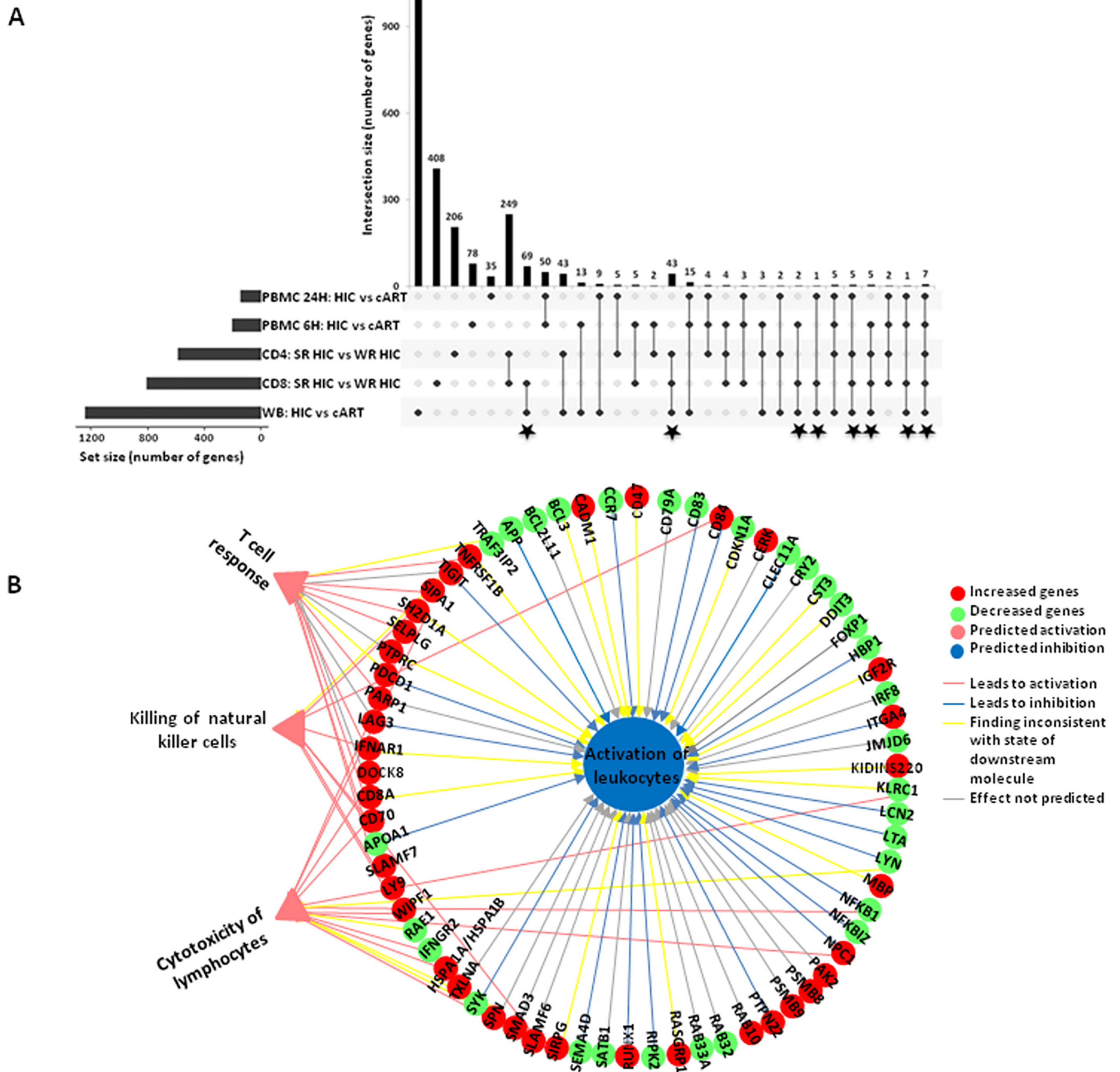

FIG 8 Summary of genes differentially expressed in the various experiments performed in the study. (A) Commonly differentially expressed genes between HIC and cART PBMC at 6 and 24 h of stimulation, between SRHIC and WRHIC CD4, between SRHIC and WRHIC CD8, and between HIC and cART in the whole blood (WB). Commonly differentially expressed genes between HIC and cART patients in WB and between SRHIC and WRHIC CD8$^+$ T cells, are indicated by stars. (B) Predicted functions committed based on the 671 genes differentially expressed specifically between SRHIC and WRHIC CD8$^+$ T lymphocytes, using Ingenuity software. Green symbols are underexpressed genes in SRHIC compared to WRHIC; red symbols are overexpressed genes. Supplementary legends are depicted in the figure.

activating KIR3DS1$^+$ and the inhibitory KIR3DL1$^+$ NK cells are increased in patients with acute HIV-1 infection in the presence of HLA-B Bw480I. However, it was not associated with reduction in HIV levels in the blood. Engagement of the inhibitory KIR3DL1 receptor on these NK cells with its ligand on the target HIV infected cells could lead to the inhibition of NK cell cytotoxicity. Similarly, studies have shown that CD56$^-$ CD16$^+$ NK cells, which are expanded in HIV-viremic individuals, have impaired function and high expression of inhibitory KIR2DL2 and KIR2DL3 recep-

tors, which would explain their defective lytic capability toward HIV-infected cells (33). Although we did not evaluate the functional capacity of NK cells in HIC, one can hypothesize that the downregulation of iKIR observed in HIC may result in strong NK cell activation, leading to viral load control.

We also observed a downregulation in HIC of receptors for the Fc portion of immunoglobulin (*FCGR3A/FCGR3B*, *FCGR2A*, and *FCER1G*). Many studies indicate that antibody-induced effectors responses mediated through FCGR signaling contribute to the control and prevention of HIV-1 infection (34–36). FCGR2A (CD32A) receptor has also been reported as a marker of the CD4$^+$ T cell HIV reservoir in HIV-infected patients (37), but more recently contradictory works have shown that CD32 is not a marker of HIV-1 reservoir but of CD4$^+$ T cell activation in HIV$^+$ individuals (38, 39). Although the role of the Fc receptors in virus control remains to be thoroughly explored, one can speculate that the downregulation of these receptors could be associated with both lower activation/inflammation and the HIV reservoir observed in HIC compared to cART-treated individuals (40). It was also reported that the quality rather than the quantity of FCGR signaling could be responsible for the wider polyfunctional Fc-mediated responses observed in HIC (36, 41). In parallel, there is a downregulation in HIC of mitogen-activated protein kinase 1 (*MAPK1*) and PI3-kinase (*PIK3CG* and *PIK3CB*); both are critical regulatory factors of immune stimulation and suppression during inflammation (15, 16, 42). In mice, an inhibition of PIKG promotes adaptive immunity and CTL activities (16, 43). Here, we observed a downregulation of PIKG associated with a downregulation of many inflammatory genes, including IL-4, especially in HIC presenting a strong viral inhibition capacity. Globally, the observation in HIC of a link between the low expression of *PIK3CG* and both an activation of T cell signaling and a downmodulation of inflammatory pathways is reminiscent of the action of this "switcher" in the balance between immune suppression and inflammation (16). HIC seem to develop an efficient adaptive immune response through a modulation of expression of regulatory molecules of the cytoplasmic signal transduction pathways *FYN*, *ZAP70*, and *MAPK1*. Indeed, an increase in the expression of Src family kinases, FYN, and ZAP70 tyrosine kinase in HIC favors the activation of T cells through the TCR, which allows a specific immune response (44, 45). This specific response was associated with a drastic downregulation of chemoattractive molecules such as *CXCL5*, *IL1B*, *IL1A*, *CCL3L1*, *CXCL1*, and *CCL24* in HIV-peptides stimulated PBMC of HIC compared to cART-treated patients. The same profile was observed with CD8 T lymphocytes from SRHIC versus WRHIC, which also have a lower proinflammatory response, through downregulation of mRNA of *CXCL8*, *S100A8*, *S100A9*, and *IL1B*, whereas the *IFNG* response was activated.

Immunological and virological aspects in the blood, gut-associated lymphoid tissues (GALT), and lymph nodes of HIC and cART patients showed the crucial role in the virus control of both HIV-specific responses and immune activation (44, 45). Our observations highlight only mechanisms involved in the blood of HIC compared to cART patients. However, HIV infection also induces the expression of different components of inflammasomes in GALT (46), and both the immune regulation and delayed progression to AIDS were associated with a particular activation phenotype of T cells in GALT from HIC (47). Furthermore, in HIV infection immune activation and inflammation were also associated with immunometabolism reprogramming through the use of glucose and fatty acids (48). In whole blood, we observed an enrichment of gluconeogenesis and lipid metabolism pathways in differentially expressed genes between HIC and cART patients, but it was not possible to determine whether there was activation or inhibition of these pathways.

Altogether, we show that HIC associate an anti-inflammatory state and strong adaptive immune response to virus that probably allows for the control of viral loads below the limits of detection. Efficient HIV therapeutic vaccine would mimic such response profiles by inducing a strong HIV-specific immune response while minimizing inflammation.

MATERIALS AND METHODS

Patients and samples. Whole-blood samples were collected from 53 HIV HIC subjects of the ANRS CO21 CODEX cohort and 27 HIV-infected cART-treated patients at Henri Mondor Hospital (Créteil, France). HIC individuals were never treated with cART, were HIV infected for at least 5 years, and had least five consecutive levels of plasma HIV RNA of $<$400 copies/ml (49). Control cART patients exhibited a plasma HIV RNA level of $<$50 copies/ml for at least 2 years and CD4 lymphocytes of $\geq$500 cells/mm^3. CD4 and CD8 T lymphocytes were purified from SRHIC and WRHIC subjects, and PBMC were obtained from HIC and cART patients. The study protocol was approved by the regional investigational review board (Comité de Protection des Personnes Ile-de-France VII and IX) with approval references 05-22 and 10-023. The study protocol was performed in compliance with the tenets of the Declaration of Helsinki.

RNA isolation and microarray sample preparation. Whole-blood RNA was purified using a Tempus spin RNA isolation kit (Thermo Fisher Scientific). PBMC, CD4, and CD8 lymphocyte RNA was purified using a Qiagen RNeasy Micro kit. RNA was quantified using a ND-8000 spectrophotometer (NanoDrop Technologies, Fisher Scientific, Illkirch Cedex, France) before being checked for integrity on a 2100 BioAnalyzer (Agilent Technologies, Massy Cedex, France). cDNA was synthesized, and biotin-labeled cRNA was generated by an *in vitro* transcription reaction using an Ambion Illumina TotalPrep RNA amplification kit (Applied Biosystems/Ambion, Saint-Aubin, France). Labeled cRNA was hybridized on Illumina Human HT-12V4 BeadChips.

CD4 and CD8 T lymphocytes isolation. CD4$^-$ and CD8$^-$ lymphocytes were isolated only from SRHIC and WRHIC subjects (9). T cells were isolated with an automated Robosep cell separator (STEMCELL) by indirect magnetic cell sorting with a T cell enrichment kit (STEMCELL) customized to also eliminate gamma/delta T cells. CD4$^+$ T cells were subsequently separated by positive selection using anti-CD4 coated beads (STEMCELL), and CD8$^+$ T cells were recovered in the resulting negative fraction. The purities of CD4 and CD8$^-$ T cells were $>$95%.

***In vitro* stimulation of purified PBMC with HIV peptides for gene expression and cytokine profile analyses.** After resting, 8×10^5 of thawed cells were stimulated for 24 h in 48-well plates with an HIV peptide pool of 36 peptides (15-mers overlapping by 11-amino-acid peptides) covering five regions of HIV Gag, Pol, and Nef (50). The cells were then pelleted for transcriptomic analysis. In parallel, 5×10^5 cells were cultured in triplicate in 96 deep-well plates and stimulated with the same antigens. On day 2, supernatants were collected for a Luminex assay. We added 100 U/ml IL-2 (Miltenyi Biotec) to the culture medium at days 2 and 5 for longer stimulation. At day 8, all wells were split in two, and cells were restimulated with the same antigens either for 6 h in the presence of brefeldin A for the ICS assay or for 24 h for the Luminex assay.

For ICS analyses, cells were first stained with surface monoclonal antibodies: anti-CD3 Alexa 700, anti-CD4 BV421 (BD Biosciences, Le Pont de Claix, France), anti-CD8 eFluor780 (Affymetrix/eBioscience, Paris, France), and a viability marker (Live/Dead Fixable Aqua Dead cell stain kit; Life Technologies, Saint Aubin, France), permeabilized, and then fixed with Cytofix/Cytoperm buffer (BD Biosciences). Cells were then stained with intracellular antibodies: anti-IFNG PerCP Cy5.5, anti-TNF-α PE-Cy7, and anti-MIP1B PE (BD Biosciences). Data were acquired with a LSRII flow cytometer (BD, Le Pont de Claix, France), with a minimum of 100,000 events collected in CD3$^+$ live cells. The data were analyzed using FlowJo software, and the specific response was expressed as the percentage of CD4 or CD8 T cells.

For the Luminex assay, 14 cytokines were measured in the supernatants of cell cultures at days 2 and 9 using Millipore reagents (Milliplex human CD8 T-cell panel with IL-2, IL-5, IL-10, IL-13, IFN-γ, TNF-α, MIP-1β, perforin, granzyme A, and granzyme B; magnetic beads and antibodies for human IP-10, IL-21, IL-17A, and IL-1β [Millipore, Chicago, IL]). Data were acquired with the Bio-Plex 200 system (Bio-Rad, Marnes-la-Coquette, France).

Statistics. Microarray data analyses were performed using R software version 3.2.2 (R Foundation for Statistical Computing, Vienna, Austria). Gene transcription data were preprocessed (51, 52) and corrected for potential batch effect (53). Statistical comparisons between groups were based on empirical Bayes moderated *t* statistics (54). An adaptive false discovery rate (FDR) procedure was used to control for test multiplicity. Unsupervised hierarchical clustering heatmap analysis was performed on raw scaling expression using a Euclidean distance matrix and Ward's linkage (55). Canonical pathway and biological function analyses were then carried out using genes differentially expressed between groups with adaptive FDR-adjusted $P \leq 0.05$ and a fold change (FC) absolute value of $\geq$1.5. Ingenuity Pathway Analysis software (Qiagen, Redwood City, CA) was used for gene pathway and function analyses. Mann-Whitney tests were used to compare cytokine production by T cells and PBMC.

Data availability. All microarray data are MIAME compliant, and the raw and normalized data have been deposited in the MIAME-compliant database Gene Expression Omnibus (GEO) under accession number GSE108297.

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H.H., C.La., J.-D.L., A.S.-C., O.L., Y.L., and R.T. contributed to study conception and design. J.-D.L. and O.L. enrolled patients from the participating hospitals and provided the clinical information. A.S.-C., H.B., E.F., H.H., C.La., O.L., C.Le., P.V., and P.T. contributed to the acquisition of data. H.B., H.H., C.La., J.-D.L., Y.L., and R.T. participated in the statistical analysis and the interpretation of data. H.H., C.La., Y.L., and R.T. contributed to the drafting of the manuscript. A.S.-C., H.B., H.H., O.L., C.La., J.-D.L., Y.L., and R.T. contributed to critical revisions.

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