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# Differential T-cell profiles determined by Hepatitis B surface antigen decrease among people with Human Immunodeficiency Virus /Hepatitis B Virus coinfection on treatment

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## Abstract

**Background** Several studies have reported that combination antiretroviral therapy (cART) enhances the hepatitis B surface antigen (HBsAg) clearance rate in Human Immunodeficiency Virus-1/Hepatitis B Virus (HIV/HBV) coinfecting patients, yet the associated immunological characteristics remain unclear.

**Methods** Global and specific immune phenotypic profiles were examined in 48 patients with HIV/HBV coinfection before cART and at 1-year, and 3-year after cART using flow cytometry. In addition, 61 patients with HBV monoinfection were included for comparison.

**Results** HBsAg response (sAg-R) was defined as >0.5 log decrease within six months of cART initiation, and 16 patients achieved it. Patients with sAg-R (the sAg-R group) exhibited distinct immune phenotypes compared to those of HBsAg-retained patients (the sAg-NR group). Notably, patients with sAg-R had lower CD4<sup>+</sup> T cell counts and a higher number of HBcAg-specific T cells. Further, the sAg-R group exhibited upregulation of HLA-DR, Ki67, and PD-1 in CD4<sup>+</sup> T cells and heightened HLA-DR and T-bet in CD8<sup>+</sup> T cells. However, the sAg-R group had fewer TEMRA cells but more TEM and Th17 cells than those in the sAg-NR group. Expression of various markers, including HLA-DR<sup>+</sup>CD4<sup>+</sup>, Ki67<sup>+</sup>CD4<sup>+</sup>, PD-1<sup>+</sup>CD4<sup>+</sup>, CD38<sup>+</sup>CD8<sup>+</sup>, HLA-DR<sup>+</sup>CD8<sup>+</sup>, TIM-3<sup>+</sup>CD8<sup>+</sup>, HBV-specific CD4<sup>+</sup> T cell secreting IFN-γ and IL-2, and specific CD8<sup>+</sup> T cell secreting IFN-γ and IL-2, correlated with HBsAg decrease.

**Conclusion** The decline in HBsAg levels during cART in HIV/HBV coinfection involves significant alterations in CD4<sup>+</sup> and CD8<sup>+</sup> T cells phenotypes, offering a novel perspective on a functional HBV cure.

**Keywords** Combination antiretroviral therapy (cART), Hepatitis B surface antigen (HBsAg), Human immunodeficiency Virus-1, Coinfection, Memory T cells, CD4<sup>+</sup> T cells, CD8<sup>+</sup> T cells

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## Background

Despite remarkable progress in preventive vaccines and antiviral therapies, chronic hepatitis B (CHB) still affects approximately 296 million people worldwide [1]. Achieving hepatitis B surface antigen (HBsAg) clearance is the ideal treatment endpoint for CHB [2, 3]. However, the HBsAg clearance rate in current nucleos(t)ide analog treated CHB remains low at 1–2% [4, 5]. HBV infections are common among patients living with human immunodeficiency virus (PLWH) worldwide [6]. Notably, several studies have reported a higher HBsAg clearance rate in patients with HIV/HBV coinfection after combination antiretroviral therapy (cART) initiation than in those with HBV monoinfection, especially with tenofovir disoproxil fumarate/ tenofovir alafenamide (TDF/TAF) treatment regimens [7–9]. While cohort studies have suggested that immune reconstitution under antiretroviral therapy may contribute to an improved HBsAg clearance rate, information on the immunological characteristics of patients with HIV/HBV coinfection is limited.

The impaired adaptive immune response plays a crucial role in the pathogenesis of persistent HBV infection [10], especially the dysfunction and exhaustion of HBV-specific T cells [11, 12]. HBsAg clearance in HBV monoinfection is partially explained by the restoration of specific T cells, increased cytokines such as IL-12, IL-17 A, and IP-10, and reduced T cell depletion [13–16]. Previous studies have demonstrated that individuals with HIV/HBV coinfection display reduced HBV-specific T-cell responses compared to those with HBV monoinfection [17, 18]. While cART promotes the recovery of CD4<sup>+</sup> T cells, the restoration of HBV-specific T cells following cART in HIV/HBV coinfection remains inconclusive [19–21]. Importantly, the absence of TDF/TAF-based cART regimens in these may account for their inability to explain higher HBsAg clearance rates observed in patients with HIV/HBV following TDF/TAF-based cART. Therefore, further investigations are required to understand the immunological features associated with HBsAg clearance in patients with HIV/HBV coinfection undergoing modern cART therapy.

To investigate the immunological mechanisms involved in HBsAg decline in patients with HIV/HBV coinfection, we performed a longitudinal analysis of the immunological profiles of patients with HIV/HBV coinfection following cART. Our focus was on comparing the immunological profiles of HIV/HBV coinfecting patients with and without rapid HBsAg decline after the cART initiation.

## Methods

### Study participants

We previously studied a cohort of 51 people living with HIV/HBV coinfection who were enrolled in the

Department of Infectious Disease at Peking Union Medical College Hospital (PUMCH) [22]. In this study, we enrolled 48 participants of these participants, with 3 excluded due to insufficient blood samples. All 48 patients were treatment-naïve for both HIV and HBV at the time of enrollment and subsequently received combination antiretroviral therapy (cART) based on 3TC or 3TC+TDF, with a follow-up period of at least six months.

For comparison of immunophenotypes, we also recruited 61 individuals with HBV monoinfection, which included 21 treatment-naïve patients (NT group) and 40 patients who were undergoing nucleos(t)ide analog (NAs) treatment (NAs group). Patients with hepatitis C coinfection, alcoholic and auto-immune liver diseases, malignancy, and those receiving Peg-IFN $\alpha$ , corticosteroids, immunosuppressants, or chemotherapeutic drugs were excluded.

### Study design

Participants with HIV/HBV coinfection were analyzed longitudinally. Routine blood tests, liver function tests, and absolute CD4<sup>+</sup> and CD8<sup>+</sup> T lymphocyte counts were determined at the PUMCH laboratory. Routine clinical data and blood samples were collected from each coinfecting participant before cART (baseline) and at the first and third years after cART initiation.

HBV monoinfection was analyzed cross-sectionally, and samples were collected at only one-time point. The immunophenotypes of treatment-naïve HBV-infected patients (NT group) were analyzed as controls for HIV/HBV patients before cART. Participants with HBV infection receiving NAs treatment (NAs group) received treatment for a median period of 12 months. Immunophenotypes of the NAs group were analyzed as the control for HIV/HBV patients at the time of 1 year after initiation of cART. The characteristics of the participants were listed in Table 1.

### Definition of HBsAg response

HBsAg response (sAg-R) was defined as a significant decrease in HBsAg level (>0.5 log decrease from baseline levels) within six months from the initiation of cART. Participants living with HIV/HBV coinfection were stratified into sAg-R and HBsAg non-response (sAg-NR) groups according to whether or not they demonstrated a sAg-R.

### Blood sample collection

Human peripheral blood mononuclear cells (PBMCs) were isolated using Ficoll density gradient centrifugation (TBD Science, Tianjin, China) and cryopreserved in liquid nitrogen for deferred use.

**Table 1** The demographic and clinical characteristics of participants

Characteristic	HIV/HBV coinfection		P-value sAg-NR vs. sAg-R	HBV mono-infection	
	sAg-NR	sAg-R		NT	NAs
Number of subjects	32	16		21	40
Age, years, mean (SD)	34.5 (7.0)	35.9 (9.0)	0.555	36.2 (9.2)	41.0 (9.7)
Male sex n (%)	29 (90.6)	15 (93.8)	1.000	17 (81.0)	36 (90.0)
<b>HBV infection parameters</b>					
HBeAg positive(%)	11 (34.4)	6 (37.5)	0.831	5 (23.8)	20 (50.0)
HBsAg level, log <sub>10</sub> IU/mL	3.7 (3.1–4.1)	3.0 (1.9–4.8)	0.498	3.4 (2.7–4.0)	3.3 (2.9–3.6)
HBV DNA, log <sub>10</sub> IU/mL	2.7 (1.5–6.6)	4.8 (2.1–8.2)	0.136	3.4 (2.7–7.1)	1.3(0-1.74)
HBV pgRNA, log <sub>10</sub> copies/ml	5.8 (3.5–6.7)	6.2 (4.5–7.4)	0.160	5.9 (3.6–7.1)	3.9 (2.9–5.2)
ALT(U/L)	26 (19–41)	23 (16–32)	0.405	30 (24–36)	23 (18–42)
Platelet count, x10 <sup>9</sup> / L	174 (43)	188 (57)	0.352	262 (132)	218 (53)
APRI score	0.39 (0.29–0.70)	0.42(0.31–0.52)	0.847	0.25 (0.17–0.33)	0.30(0.23–0.40)
FIB-4 score	1.03 (0.75–1.84)	1.52(0.83–1.71)	0.516	0.68 (0.34–1.20)	0.95(0.69–1.37)
<b>HIV Infection parameters</b>					
HIV RNA, log <sub>10</sub> copies/ml	4.6 (3.8-5.0)	4.9 (4.7–5.6)	0.088	n.d.	n.d.
HIV DNA, log10 cps/ml/10 <sup>6</sup> WBC	3.2 (2.7–3.4)	3.1 (2.9–3.5)	0.981	n.d.	n.d.
CD4 T cells counts	258 (120)	157 (100)	<b>0.006</b>	n.d.	n.d.
CD8 T cell counts	822 (324)	827 (544)	0.967	n.d.	n.d.
CD4/CD8 ratio	0.32 (0.16)	0.25 (0.21)	0.206	n.d.	n.d.

Note: Data are presented as mean(standard deviation), the number of patients(percent) or median(Inter quartile range). Differences in variables were analyzed using the Paired-Samples T-Test,  $\chi^2$  tests, and Wilcoxon matched-paired signed-ranked test, respectively

Abbreviations: sAg-R: sAg-NR HIV, human immunodeficiency virus; HBV, hepatitis B virus; HBeAg, hepatitis B envelope antigen; HBsAg, hepatitis B surface antigen; ALT, alanine aminotransferase; APRI, AST to Platelet Ratio index; FIB-4, Fibrosis-4

### Serological assays

Hepatitis B surface antigen (HBsAg), anti-HBs, hepatitis B e-antigen, anti-HBe, and anti-HBc levels were detected using an Abbott Architect i2000 SR immunoassay analyzer (Abbott Diagnostics, Illinois, USA). HBsAg levels were quantified using the Abbott Architect i2000 platform.

### HIV-1 total DNA measurement

HIV-1 total DNA levels were quantified using the HIV-1 DNA Quantification kit (Supbio, Guangzhou, China) on the ROCHE RT-qPCR platform, as previously described [23].

### Phenotypic analysis using flow cytometry

To explore the phenotype of T cells, frozen PBMCs were thawed and rested overnight, then were incubated with Fixable Viability Stain 700 (FVS700; BD Biosciences, San Jose, USA). Surface staining was performed for 30 min at 4°C. For detection of intranuclear proteins, fixation and permeabilization (562574#, BD Biosciences) were performed before staining with intracellular antibodies. The antibodies used for phenotypic analysis are listed in Table S1. To perform intracellular cytokine staining (ICS) on global T cells, PMA (2 ng/mL; Beyotime, Shanghai, China), Ionomycin (1 µg/mL; Beyotime) and Glogistop Protein Transport Inhibitor (BD Biosciences) were added for 6 h following cell resuscitation. Surface and

intracellular staining were performed as described above. A minimum of 100,000 events were acquired per sample tube by using a Fortessa LSR flow cytometer (BD Biosciences). Flow cytometry data were analyzed using FlowJo™ software version 10 (BD, Ashland, OR, USA). Single compensation tubes and fluorescent minus-one (FMO) tubes were used as controls. The analysis strategy for T cells and their subsets is illustrated in Figure S1-S3.

### HBV-Specific T cell response experiments

Thawed PBMCs were stimulated with HBcAg overlapping peptides (genotypes B and C, GenBank accession numbers: AF121243 and AF112063, Genescript) for 10 days in R10. Thirty-seven core-specific peptides, 20 U/ml of human recombinant IL-2 (rhIL-2; R&D system, Bio-Techne, Minneapolis, MN, USA), 20 U/ml of human recombinant IL-7 (R&D system, Bio-Techne), 0.5 µg/mL of CD28/CD49a (BioLegend, San Diego, CA, USA) were used for stimulation and expansion on day 0. The culture was changed and replaced with half the original volume and supplemented with rhIL-2 on days 3 and 7. On day 10, PBMCs were restimulated for 6 h with the HBV core-peptide pool. Glogistop (BD Biosciences) was then used for 5 h. Then PBMCs were stained and tested for expression of IFN- $\gamma$ , TNF- $\alpha$ , and IL-2 using flow cytometry analysis.

### RNA sequencing and functional enrichment analysis

To analyze the differential gene expression profile determined by the HBsAg decline rate in patients with HIV/HBV coinfection, total RNA was extracted from PBMC using TRIzol (Invitrogen Life Technologies, Carlsbad, CA, USA), and samples were shipped frozen to Biomarker Technologies (Beijing, China) for transcriptome sequencing. Transcriptome sequencing was performed using a HiSeq 2500 platform (Illumina, San Diego, CA, USA). Differential expression analyses were performed using the BMKCloud platform (<https://www.biocloud.net>).

### Statistical analysis

Statistical significance was determined using GraphPad Prism version 8.3 (GraphPad Software, Inc., CA, USA) or SPSS version 26 (IBM, Chicago, IL, USA) using the tests stated in the figure legends. Significant differences between the two groups were determined using the Mann-Whitney U test. Significant differences among three or more groups were determined using a mixed-effects model. Correlations between variables were determined using Spearman's rank correlation test. The radar plot was depicted using Origin version 10.0 (Northampton, MA, USA), and a heat map was generated using Chiplot (<https://www.chiplot.online>). All reported P-values were two-sided, and a P value less than 0.05 was considered statistically significant.

## Results

### Characteristics of patients

Forty-eight patients with HIV/HBV coinfection were included in this study, none of whom had prior exposure to HIV or HBV treatment. Nineteen patients were analyzed for HBV genotypes: thirteen were genotype C and six were genotype B. Thirty-eight patients (81.3%) were started on a 3TC+TDF-based antiviral regimen, nine patients received 3TC-based antiviral regimens, and one patient received a TAF/FTC-based antiviral regimen. After the initiation of cART, 16 patients achieved more than 0.5 log<sub>10</sub> IU reduction in HBsAg levels from baseline within six months (HBsAg response group, sAg-R group), and 32 patients had HBsAg levels comparable to baseline during follow-up, considered to be in the HBsAg non-response group (HBsAg non-response group, sAg-NR group). In addition, patients with hepatitis B Virus patients (NAs and NT group) were included as controls. The demographic and clinical characteristic profiles of participants are provided in Table 1. At baseline, no differences were detected in HBsAg status, HBsAg level, HBV DNA, HBV pgRNA, and ALT levels between the sAg-R and sAg-NR groups.

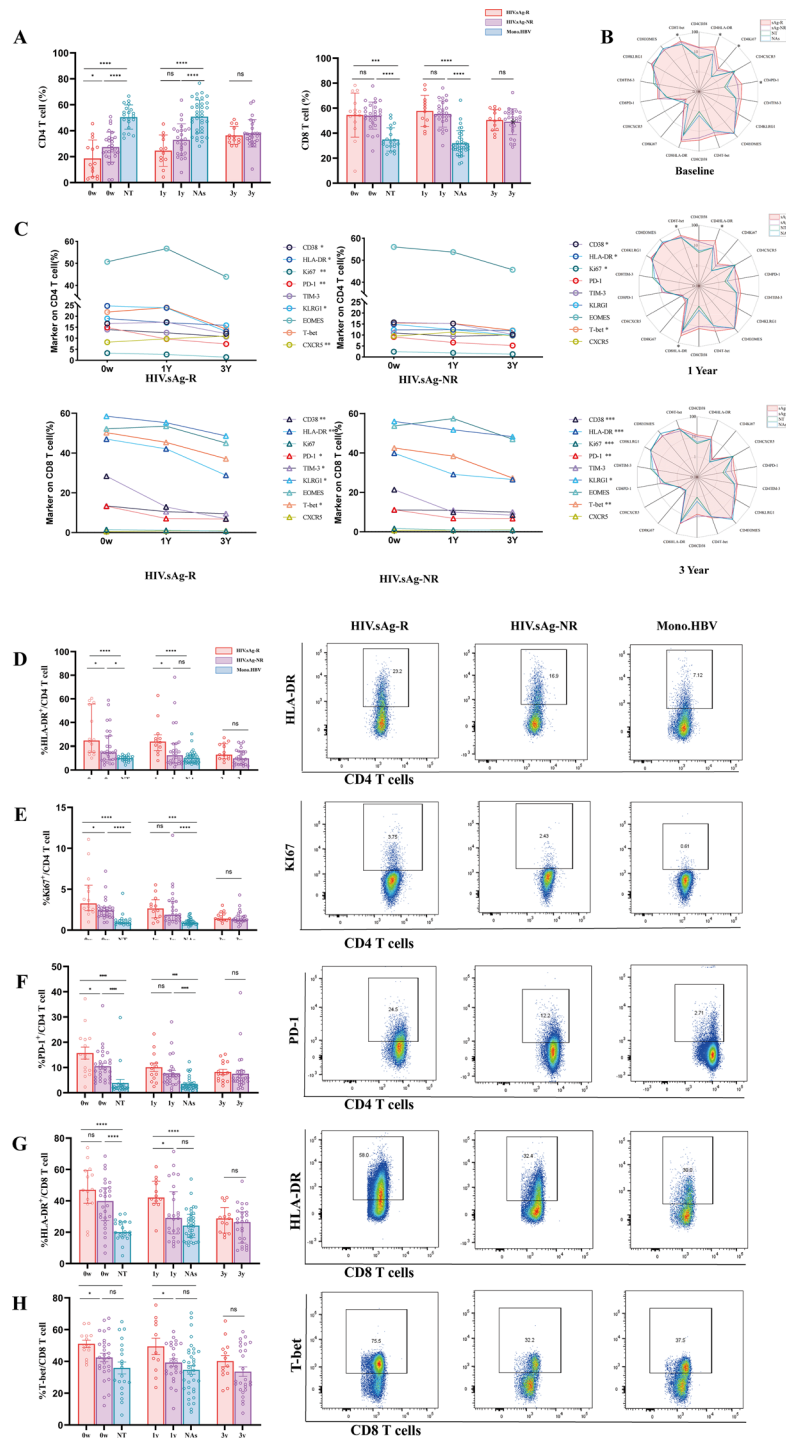
### Differential expression of activation and exhaustion markers in patients with different rates of HBsAg decline

Flow cytometry analysis showed that the proportion of CD4<sup>+</sup> T cells in the sAg-R group was significantly lower than that in the sAg-NR group at baseline but recovered by the end of the third year (Fig. 1A). To understand the alteration of the immunophenotype during HBsAg clearance, we analyzed the expression levels of activation markers CD38 and HLA-DR; proliferation marker Ki67; exhaustion markers PD-1, TIM-3, and KLRG1; transcription factors T-bet and EOMES; and chemokine receptor CXCR5 in T cells using flow cytometry. As shown in Fig. 1B and Figure S4, HIV/HBV coinfection upregulated the expression of several activation and exhaustion markers compared to HBV mono-infection, such as CD38, HLA-DR, Ki67, PD-1, TIM-3, and T-bet on CD4<sup>+</sup> T cells and CD38, HLA-DR, Ki67, PD-1, KLRG1, and T-bet on CD8<sup>+</sup> T cells, whereas CXCR5, TIM-3, and EOMES were downregulated on CD8<sup>+</sup> T cells (Fig. 1B). After initiation of cART, the expression levels of CD38, HLA-DR, Ki67, PD-1, TIM-3, KLRG1, and T-bet decreased in coinfecting patients, whereas CXCR5 increased in sAg-R patients (Fig. 1C), indicating that antiviral treatment and suppression of viral load improved the immune exhaustion status among coinfecting patients.

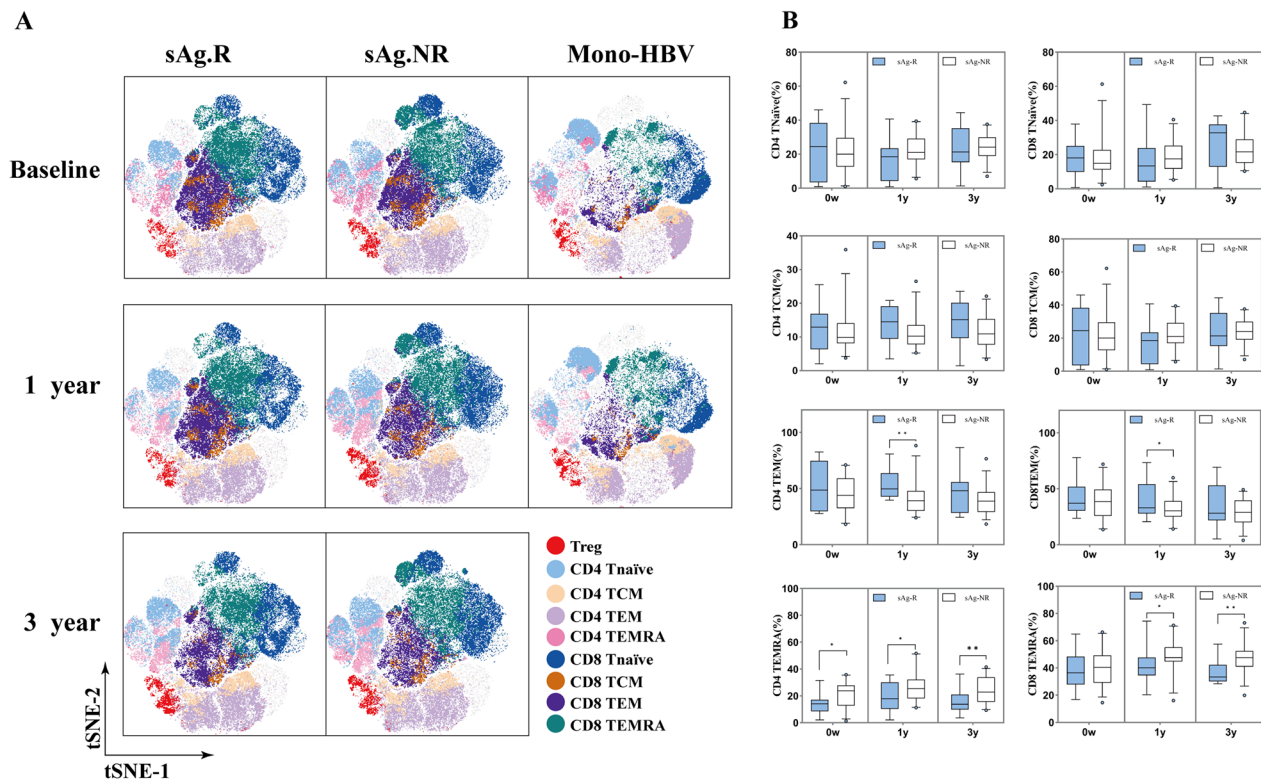
Notably, a different T cell immunophenotype was observed in the sAg-R patients compared to the sAg-NR patients. CD4<sup>+</sup> T cells from the sAg-R group had significantly higher expression of HLA-DR (Fig. 1D; at baseline: 23.80% vs. 12.50%,  $P=0.0111$ ; at the first-year follow-up assessment: 24.9% vs. 14.9%,  $P=0.0300$ ), Ki67 (Fig. 1E; at baseline: 3.3% vs. 2.4%,  $P=0.0228$ ), and PD-1 (Fig. 1F; at baseline: 14.9% vs. 9.1%,  $P=0.0357$ ) than those in the sAg-NR group. The expression of HLA-DR (Fig. 1G; at the first-year follow-up assessment: 42.2% vs. 29.1%,  $P=0.0258$ ) and T-bet (Fig. 1H; at baseline: 51.0% vs. 42.5%,  $P=0.0491$ ; at the first-year follow-up, 49.5% vs. 39.3%,  $P=0.0440$ ) on CD8<sup>+</sup> T cells in the sAg-R group was also significantly increased compared to those in the sAg-NR group. Taken together, these data demonstrated that the sAg-R group had higher T-cell activation and proliferation levels at baseline and longitudinally.

### Increased effector memory T cell subsets in patients with rapid HBsAg decline

The differentiation memory phenotype was detected using CD45RA and CCR7 staining, followed by t-SNE and conventional flow cytometry analysis (Fig. 2). As expected, the t-SNE plot showed that the altered T subsets of HIV/HBV coinfection demonstrated higher proportions of CD8<sup>+</sup> subsets and CD8<sup>+</sup> effector memory T cells (CD45RA<sup>-</sup>CCR7<sup>-</sup>, TEM), and lower proportions of naive T cells (CD45RA<sup>+</sup>CCR7<sup>+</sup>, Naive) compared to those of HBV mono-infection. In contrast to the dramatic



**Fig. 1** Expression of activation and exhaustion markers in patients with different rates of HBsAg decline. **(A)** Bar chart displays the median frequency of CD4<sup>+</sup> and CD8<sup>+</sup> T cells in HIV/HBV coinfecting patients with HBsAg response (HIV.sAg-R) and non-response (HIV.sAg-NR) at baseline, 1 year, and 3 years after starting treatment, as well as treatment-naïve chronic hepatitis B virus-infected patients (NT) and nucleos(t)ide analogue (NAs)-treated hepatitis B virus patients (NAs Group). The Mann-Whitney test was used for analysis. **(B)** Radar plots illustrate the median expression of activation and exhaustion markers on CD4<sup>+</sup> and CD8<sup>+</sup> T cells for HIV.sAg-R, sAg-NR patients, NT, and NA patients. **(C)** Median marker expression on CD4<sup>+</sup> and CD8<sup>+</sup> T cells of sAg-R and sAg-NR patients at baseline, 1 year, and 3 years after starting treatment. Mixed-effect analysis was employed. **(D-H)** The median expression of HLA-DR<sup>+</sup> CD4<sup>+</sup>, Ki67<sup>+</sup> CD4<sup>+</sup>, PD-1<sup>+</sup> CD4<sup>+</sup>, HLA-DR<sup>+</sup> CD8<sup>+</sup>, and T-bet<sup>+</sup> CD8<sup>+</sup> in participants, along with representative flow cytometry analyses  
\**p* < 0.05; \*\**p* < 0.01; \*\*\**p* < 0.001; \*\*\*\**p* < 0.0001. Abbreviation: ns, not significant



**Fig. 2** Characterization of Memory T Cell Subsets in HBV and HIV/HBV Coinfected Patients. **(A)** t-SNE plots illustrating differences in CD4<sup>+</sup> and CD8<sup>+</sup> memory T cell populations at baseline, 1 year, and 3 years after treatment initiation. **(B)** Floating bars displaying the minimum and maximum values with medians for memory T cell subsets in HIV.sAg-R and sAg-NR patients at baseline, 1 year, and 3 years after starting treatment. Unpaired t-tests and Mann-Whitney tests were utilized for statistical analysis

\* $p < 0.05$ ; \*\* $p < 0.01$ ; \*\*\* $p < 0.001$ ; \*\*\*\* $p < 0.0001$

alterations in T cell subsets between coinfection and monoinfection, only minor changes were observed when the phenotype was characterized by different rates of HBsAg decline (sAg-R vs. sAg-NR group) following cART initiation (Fig. 2A). Further, the proportions of naïve T cells, TCM cells, and Tregs were similar between the sAg-R and sAg-NR patients. However, sAg-R patients had significantly higher frequencies of CD4<sup>+</sup> TEM cell subsets after one year of cART and lower frequencies of CD4<sup>+</sup> terminally differentiated effector memory T cells (CD45RA<sup>+</sup>CCR7<sup>-</sup>, TEMRA) before and during cART compared to sAg-NR patients (Fig. 2B). For CD8<sup>+</sup> T cells, sAg-R patients also had significantly higher frequencies of TEM cell subsets and lower TEMRA than sAg-NR patients after one year of cART (Fig. 2B). These results suggested that HBV strongly impacts the differentiation of memory T cells.

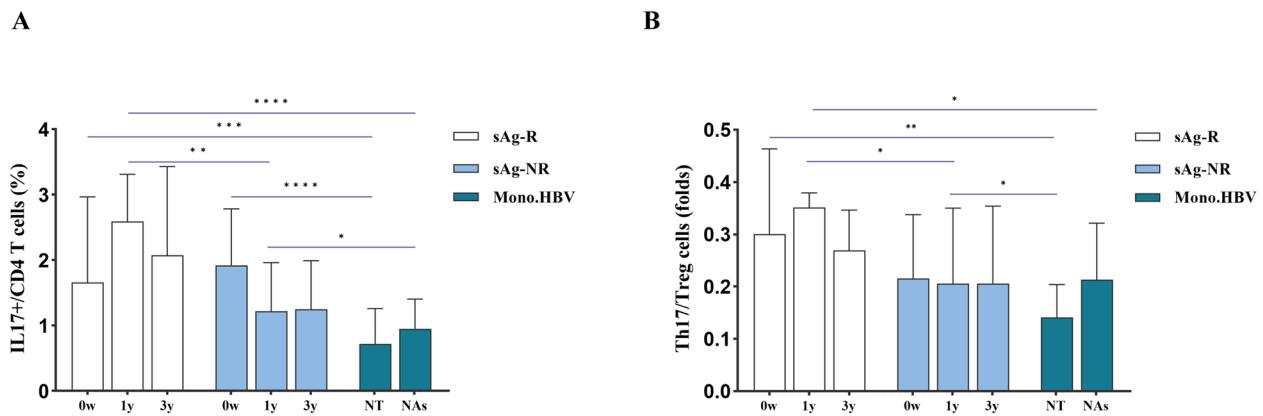
#### Elevated IL17A and Th17/Treg ratio in patients with rapid HBsAg decline

Global T cell cytokines reflect the magnitude of inflammation and immune activation and contribute to viral control. To understand the extent of HBsAg clearance in HIV/HBV-coinfected patients, we evaluated the

frequencies of global T cell-producing cytokine profiles in HIV/HBV coinfecting and HBV monoinfecting patients (Figure S5). Statistically significant differences were observed in the frequency of CD4<sup>+</sup> T cells producing IL-17 between the sAg-R and sAg-NR groups during cART, with a higher frequency in the sAg-R than in the sAg-NR group (Fig. 3A, first year of cART: 2.6% vs. 1.2%,  $P = 0.0033$ ). According to the results of IFN- $\gamma$ , IL-4, and IL-17 A staining, cell subsets were divided into Th1 cells (CD4<sup>+</sup> IFN- $\gamma$ <sup>+</sup>, Th2 cells (CD4<sup>+</sup> IL-4<sup>+</sup>), and Th17 cells (CD4<sup>+</sup> IL-17 A<sup>+</sup>). As Fig. 3B showed, the Th17/Treg ratios were more imbalanced in the HIV/HBV coinfection group than in the HBV monoinfection group. Notably, the sAg-R group had a significantly higher Th17/Treg ratio than the sAg-NR group (0.35 vs. 0.21,  $P = 0.0463$ ). Increased IL-17 levels and Th17/Treg ratios indicated an inflammatory response in patients with sAg-R.

#### Kinetic analysis of HBV-specific T cells in patients with HIV/HBV coinfection

We conducted a longitudinal analysis of HBV-specific CD4<sup>+</sup> and CD8<sup>+</sup> T cell responses in patients with HIV/HBV coinfection. As shown in Fig. 4A, the proliferation level (measured by Ki67 expression) of HBV-specific



**Fig. 3** The IL17 secreting CD4 T cell and the Th17/Treg in Patients with Different Rates of HBsAg Decline. **(A)** Summary bar charts display the median with interquartile range of IL17 secreting CD4 T cell levels; and **(B)** the median with interquartile range of Th17/Treg ratio in NT, NAs, and HIV/HBV coinfecting participants at baseline, 1 year, and 3 years after treatment initiation. Mann-Whitney tests were used for statistical analysis  
\* $p < 0.05$ ; \*\* $p < 0.01$ ; \*\*\* $p < 0.001$ ; \*\*\*\* $p < 0.0001$

CD4<sup>+</sup> T cells was significantly lower in HIV/HBV coinfection compared to HBV monoinfection, both pre- and post-antiretroviral treatment. Within coinfecting patients, the sAg-R group exhibited a lower proliferation level of HBV-specific CD4<sup>+</sup> T cells than the sAg-NR group, although the difference was not statistically significant. Additionally, the proliferative capacity of CD4<sup>+</sup> T cells responding to HBcAg was restored in the sAg-R group. We also assessed the cytokine-secreting T cells that responded to HBV core peptides. We observed that HBV core-specific CD4<sup>+</sup> T cells in HIV/HBV coinfecting patients secreted lower IFN- $\gamma$  but a higher IL-2 than HBV monoinfection. Interestingly, in the first year, the sAg-R group displayed higher levels of IFN- $\gamma$  (3.2% vs. 1.5%,  $P = 0.0131$ ) and IL-2 (1.72% vs. 1.06%,  $P = 0.0408$ ) compared to the sAg-NR group.

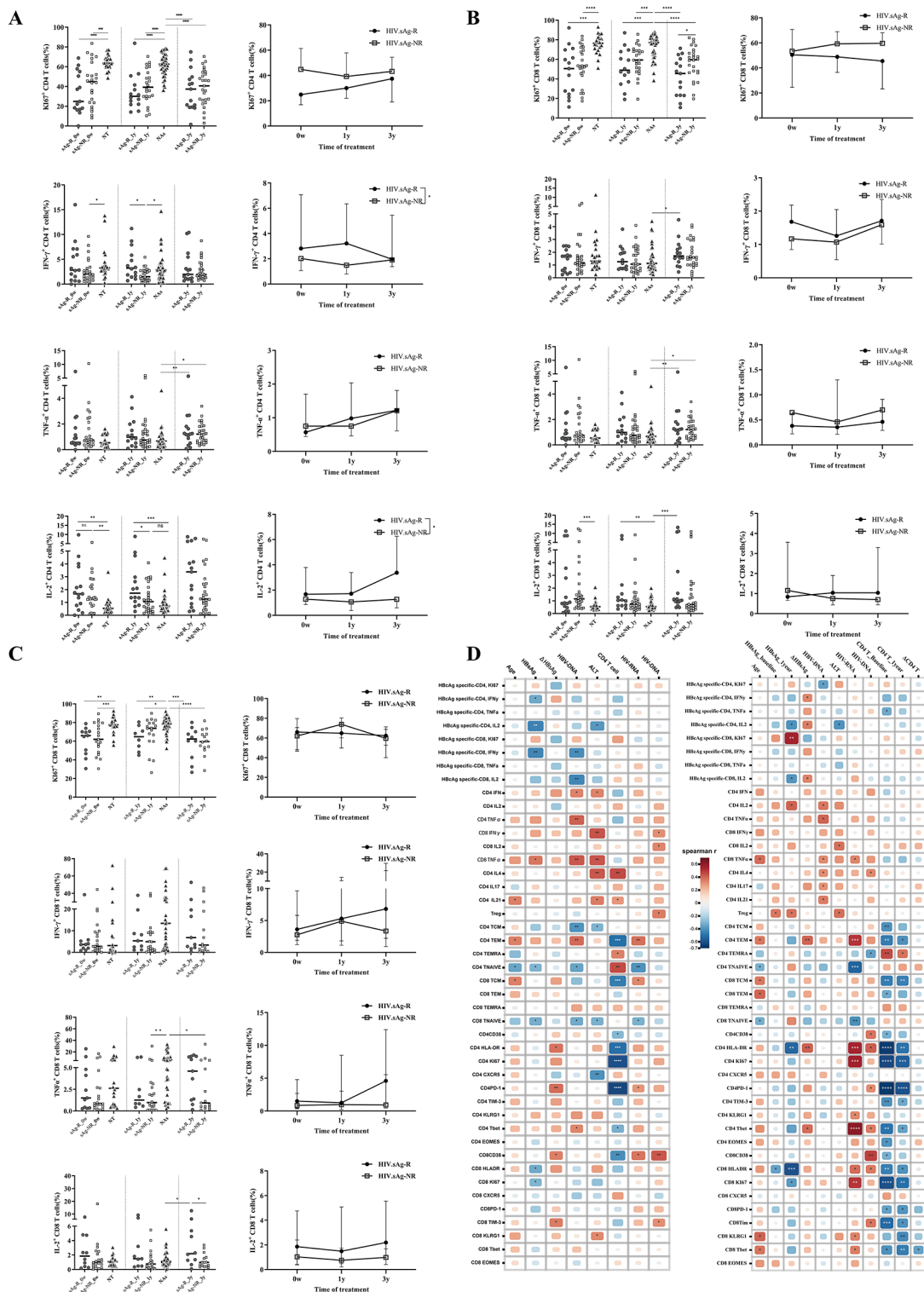
To assess specific CD8<sup>+</sup> T cells, they were analyzed using PBMCs stimulated with CEF peptides as controls (Fig. 4B and C). The proliferative capacity of specific CD8<sup>+</sup> T cells was lower in HIV/HBV coinfecting patients than in HBV monoinfecting patients, regardless of stimulation by HBV core or CEF peptides. CEF-specific CD8<sup>+</sup> cells secreted significantly higher levels of IFN- $\gamma$  and TNF- $\alpha$  in HBV-monoinfecting patients than in HIV/HBV-coinfecting patients. However, the secretion of IFN- $\gamma$  and TNF- $\alpha$  by HBV core-specific CD8<sup>+</sup> cells did not differ between HIV/HBV coinfection (sAg-R and sAg-NR) and monoinfection, both remaining at low levels. This result suggests that HIV infection affected the CD8<sup>+</sup> cell response to CEF but did not further impair the poor specific CD8<sup>+</sup> cell response to HBV. In addition, no recovery of specific CD8<sup>+</sup> cell proliferation or secretory functions was observed. The results suggest that the HBsAg decline in HIV/HBV coinfecting patients might be linked more closely to HBV-specific CD4<sup>+</sup> T cells than CD8<sup>+</sup> T cells.

#### Association between clinical characteristics of patients and T cell phenotype alterations

We investigated potential correlations between the T cell phenotype and clinical markers in HIV/HBV-coinfecting patients using Spearman's rank correlation test. Figure 4D illustrates that baseline level of HBV-specific CD4<sup>+</sup> secreting IFN- $\gamma$  (OR=-0.341,  $P = 0.032$ ), IL-2 (OR=-0.452,  $P = 0.003$ ), and specific CD8<sup>+</sup> secreting IFN- $\gamma$  (OR=-0.442,  $P = 0.003$ ) were negatively correlated with HBsAg levels. After 1-year of treatment, levels of specific CD4<sup>+</sup> secreting IL-2 (OR=-0.406,  $P = 0.021$ ) and specific CD8<sup>+</sup> secreting IL-2 (OR=-0.396,  $P = 0.025$ ) remained negatively correlated with the HBsAg. This result suggested that HBsAg levels may suppress host HBV immunity both pre- and post-treatment. Moreover, positive associations were observed between HBV-specific T-cell responses and HBsAg decline post-treatment. HBV-specific CD4<sup>+</sup> secreting IFN- $\gamma$  (OR=0.339,  $P = 0.028$ ) and IL-2 (OR=0.352,  $P = 0.022$ ), and specific CD8<sup>+</sup> secreting IFN- $\gamma$  (OR=0.292,  $P = 0.061$ ) and IL-2 (OR=0.338,  $P = 0.029$ ) were positively associated with HBsAg decline. Furthermore, the expression levels of HLA-DR<sup>+</sup> CD4<sup>+</sup>, Ki67<sup>+</sup> CD4<sup>+</sup>, PD-1<sup>+</sup> CD4<sup>+</sup>, CD38<sup>+</sup> CD8<sup>+</sup>, HLA-DR<sup>+</sup> CD8<sup>+</sup>, TIM-3<sup>+</sup> CD8<sup>+</sup> were positively correlated with HBsAg decline.

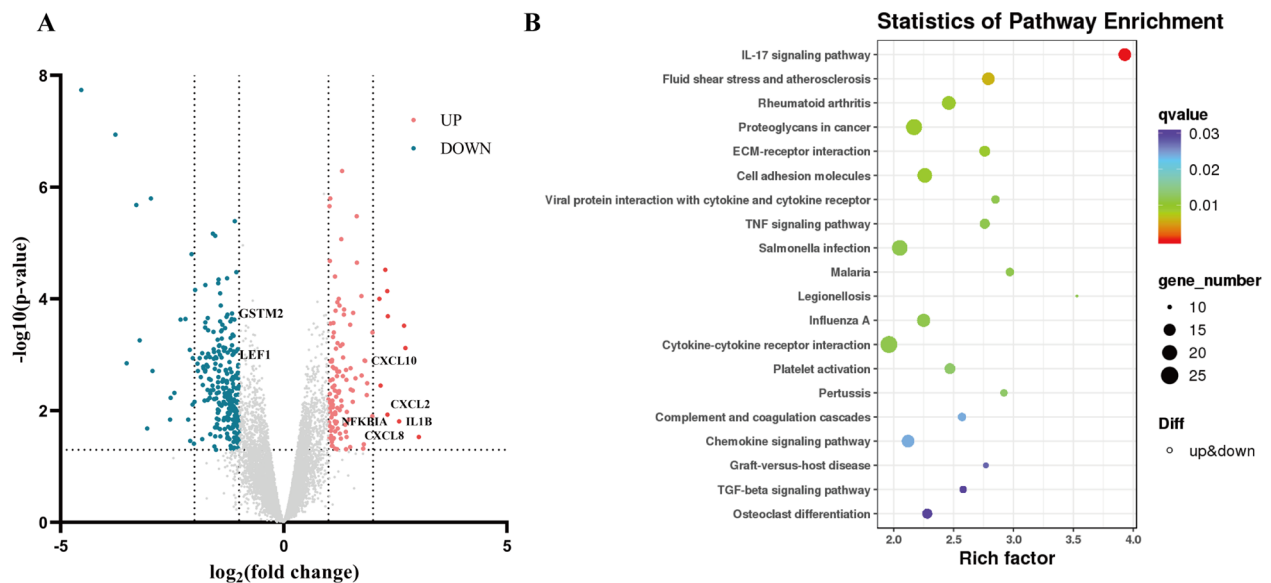
#### RNA transcription

To understand the immune alterations during HBsAg decline in coinfecting patients, mRNA sequencing analysis was performed on the PBMC of 6 sAg-R and 9 sAg-NR patients, one year after cART initiation. A total of 120 genes were upregulated, and 271 genes were downregulated in the sAg-R group compared to the sAg-NR group (Fig. 5A, Table S2). A KEGG mapper was used for pathway enrichment analysis (Fig. 5B), which revealed an



**Fig. 4** Kinetic Analysis of HBV-Specific T Cells and Relationship Between Clinical Characteristics of Patients and Altered T-Cell Phenotype. The phenotypes of HBcAg-specific CD4<sup>+</sup> (A), CD8<sup>+</sup> T (B), and CEF-specific CD8<sup>+</sup> T (C) cell responses were longitudinally analyzed. On the left, the frequency of specific cytokine-producing T cells is shown, while on the right, kinetic changes in specific T cell responses are demonstrated at indicated time points for sAg-R and sAg-NR patients. Horizontal bars depict median values. Mann-Whitney U tests were used for statistical analysis. \* $p < 0.05$ ; \*\* $p < 0.01$ ; \*\*\* $p < 0.001$ ; \*\*\*\* $p < 0.0001$ . (D) Association between the clinical characteristics of patients and altered T cell phenotypes. On the left, this association is examined at baseline, and on the right, at 1 year after treatment. Spearman's Rank correlation test was utilized





**Fig. 5** RNA-Seq and KEGG Analysis between sAg-R and sAg-NR Groups. **(A)** The volcano chart displays differentially expressed genes (DEGs) identified by RNA-Seq between sAg-R and sAg-NR groups. DEGs are defined as those with a p-value < 0.05 and a fold change (FC) > |1.5|. **(B)** The top-enriched pathways following KEGG analysis. The y-axis represents the pathways, and the horizontal axis indicates the enrichment factor. The color of the circle corresponds to the q value, which is the multiple hypothesis-corrected p-value. The size of the circle reflects the number of genes enriched in the pathway

upregulation of the IL-17 signaling pathway in patients with sAg-R.

## Discussion

Recently, the phenomenon of HBsAg seroclearance in HIV/HBV coinfection following cART has received greater attention. This is primarily due to the noted higher rates of HBsAg seroclearance in coinfections than in cases of HBV mono-infections. Despite this emerging focus, there remains a notable gap in our understanding of the specific cellular immune responses in this context. We conducted a comprehensive longitudinal study of T cell phenotypic profiles and HBV-specific immune responses in patients with HIV/HBV coinfection three years after initiating cART. Our data suggest that the rapid HBsAg decline observed in coinfecting patients is associated with T cell activation, especially in CD4<sup>+</sup> T cells, along with changes in the frequency of TEM, Th17 cell populations, and HBV-specific CD4<sup>+</sup> T cell responses.

Our findings of increased expression of HLA-DR<sup>+</sup> CD4<sup>+</sup>, Ki67<sup>+</sup> CD4<sup>+</sup>, and HLA-DR<sup>+</sup> CD8<sup>+</sup> in HBsAg decline, which suggest that the rapid decrease in HBsAg is associated with T cell activation in HIV/HBV coinfection. Consistent with our finding, a recent study measuring the T cell phenotype of patients with CHB with rapid HBsAg decrease and HBsAg seroclearance showed that the activated T cell phenotype was positively correlated with the degree of HBsAg decline [15]. Further, Rinker et al. revealed that patients with HBsAg loss had a higher

T cell-activated phenotype than patients with retained HBsAg after structural drug discontinuation [24].

Furthermore, we observed a higher proportion of TEM and Th17 subsets but a lower TEMRA subpopulation in HIV/HBV coinfecting patients with rapid HBsAg decline than in the sustained HBsAg group. TEM cells could respond rapidly to reinfection by producing effector cytokines, important for viral clearance [25]. A recent study by Islam et al. also reported an increased proportion of the TEM population in patients with HBsAg seroclearance in CHB [13]. Th17 cells play a dual role in viral clearance and liver inflammation during chronic HBV infection by eliciting the cytokines IL-17 and IL-21<sup>26</sup>. The higher levels of TEM and Th17 cells in the HBsAg-responsive group suggest that these cells may also play a role in HBsAg decline during HIV/HBV coinfection.

The correlation analysis indicated that HBsAg decline was associated with the level of HBV-specific CD4<sup>+</sup> T cells secreting IFN- $\gamma$  and IL-2, as well as HBV-specific CD8<sup>+</sup> T cell secretion of IFN- $\gamma$ . Moreover, after one year of treatment, we observed a higher level of HBV-specific CD4<sup>+</sup> T cells secreting IFN- $\gamma$  and IL-2 in coinfecting patients within the sAg-R group. These findings suggest a connection between HBsAg clearance and HBV-specific CD4<sup>+</sup> cell responses. Notably, conflicting data exists regarding the recovery of HBV-specific T cells in HIV/HBV-coinfecting patients after the initiation of treatment [19, 27–29]. In this study, we observed the recovery of the proliferative capacity of HBV-specific T cells post-treatment without a concurrent increase in the overall

quantity of cytokines secreted by specific T cells. However, the frequency of HBV-specific T cells in the peripheral blood remained low in both HIV/HBV coinfection and HBV mono-infection, with CEF-specific responses being higher in HBV mono-infected patients than in those with HIV/HBV coinfection.

In transcriptomic studies of patients with CHB, interferon-stimulated genes, immune activation/depletion genes and chemokine genes were significantly upregulated during the immune clearance phase [30, 31]. Our DEG analysis showed that patients in the sAg-R group exhibited an upregulated expression of chemokine and inflammatory factor genes enriched in the IL-17 signaling pathway compared to the sAg-NR group. This finding suggests that patients with HBsAg-responsive coinfection display characteristics indicative of the immune clearance phase.

Based on these findings, we speculate that immune activation and HBV-specific CD4<sup>+</sup> cells may contribute to the clearance of HBsAg in coinfecting patients. Previous studies have shown that patients who achieve HBsAg loss during NA therapy tend to have higher levels of HBV-specific CD4<sup>+</sup> T cells compared to HBsAg-positive patients [32]. Additionally, HBV env-specific CD4<sup>+</sup> T cells are linked to HBsAg loss following therapy withdrawal [33]. Theoretically, CD4<sup>+</sup> T cells can enhance HBV-specific CD8<sup>+</sup> T responses and facilitate HBsAb production by B cells [34]. Therefore, therapeutic strategies aimed at enhancing HBV-specific CD4<sup>+</sup> T cell responses may increase the likelihood of achieving higher rates of HBsAg clearance. Moreover, in two preclinical mouse models of HBV carriers, the activation status of HBV-specific CD4<sup>+</sup> T cells was critical in determining the success of therapeutic hepatitis B vaccination [35]. Thus, interventions targeting the enhancement of CD4<sup>+</sup> T cell activation and response could contribute to the functional cure of HBV.

This study has several limitations. First, we did not measure the immunological phenotype of HIV/HBV coinfecting patients at 12 and 24 weeks post-treatment, which may have led to missing important immunological changes during the early phase of treatment. Second, our study lacks liver tissue samples, preventing a comparative analysis of the phenotypic characteristics between peripheral blood and liver immune cells. Additionally, we did not include patients with acute HIV infection, which restricts our ability to evaluate the dynamics of HBsAg clearance and the corresponding immune phenotypes in chronic HBV-infected individuals who initiate treatment during the acute phase of HIV infection.

In summary, our findings offer a comprehensive analysis of immunophenotypic changes in HIV/HBV coinfecting patients, suggesting that post-treatment immunophenotypic changes in coinfecting patients may be associated with HBsAg clearance. This study provides crucial insights into the role of immune activation and HBV-specific CD4<sup>+</sup> T

cells as a functional cure for HBV infections. Our findings may partially explain the higher HBsAg seroclearance rate in coinfecting patients.

### Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1186/s12967-024-05681-y>.

Supplementary Material 1  
Supplementary Material 2  
Supplementary Material 3  
Supplementary Material 4  
Supplementary Material 5  
Supplementary Material 6  
Supplementary Material 7

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### Author contributions

Xiaodi Li: Conceptualization(equal); data Curation(equal), formal analysis(equal), investigation(equal), methodology (equal), visualization(equal), resources (equal); software (equal); writing – original draft; writing-review and editing (equal). Ling Xu, Lianfeng Lu, Xiaosheng Liu, Yang Yang, and Yuanni Wu: formal analysis (equal); methodology (equal); validation (equal); visualization(equal); software (equal) writing – review and editing (equal). Ting Zhu, Xiaoxia Li, Yanling Li, Xiaojing Song, Yang Han, Wei Lyu: Data curation (equal); formal analysis (equal); investigation(equal), validation(equal); writing – review and editing (equal). Wei Cao, Taisheng Li: conceptualization(equal); data curation(equal); formal analysis (equal); funding acquisition(equal); investigation (equal); methodology (equal); project administration(equal); supervision(equal); validation (equal); writing – Original draft preparation; writing- review and editing (equal).

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### Data availability

The original contributions presented in this study are included in the article and supplementary material. Further inquiries can be directed at the corresponding author.

### Declarations

#### Ethics approval and consent to participate

This study was conducted in compliance with the Declaration of Helsinki and approved by the Institutional Review Board of PUMCH. Written informed consent was obtained from all participants.

#### Competing interests

The authors declare that they have no competing interests.

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