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Dysfunction of circulating follicular helper T cells in people living with HIV who are HBV small surface protein antibody (HBsAb)-negative despite HBV vaccination

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Abstract

Background The seroconversion rate after hepatitis B virus (HBV) vaccination in people living with human immunodeficiency virus (HIV) is reportedly lower than that in non-HIV controls. Follicular helper T (Tfh) cells play a central role in humoral immunity, and IL-21 secreted by Tfh cells is essential for B cells to differentiate into plasma cells. This study investigated the frequency and function of circulating Tfh (cTfh) cells isolated from people living with HIV (PWH) who are negative for hepatitis B virus (HBV) surface antigen (HBsAb−) in a small cohort of PWH who received HBV vaccination.

Case presentation The frequency of cTfh cells in HBsAb− PWH (HBsAb levels less than 10 mIU/mL) was the same as that in non-HIV controls and HBsAb positive (HBsAb+) PWH, who maintained HBsAb at 10 mIU/mL for at least 1 year after receiving three doses of the HBV vaccine. However, after immune stimulation with anti-CD3/CD28 antibodies, the frequency of cTfh cells in the non-HIV controls and HBsAb+ PWH increased, whereas the frequency of cTfh cells in the HBsAb− PWH tended to be more gradual. cTfh17-like and cTfh2-like cells, subsets of cTfh cells, are involved in humoral immunity, and PD-1 regulates the function of these cells in the germinal center. The frequencies of PD-1+ cTfh17-like and cTfh2-like cells at baseline did not differ significantly among the three groups. However, HBsAb− PWH had a lower frequency of PD-1+ cTfh17-like cells after immune stimulation than non-HIV controls did. The frequencies of PD-1+ cTfh17-like cells and cTfh2-like cells with high PD-1 expression were significantly lower in HBsAb− PWH than in non-HIV controls. Furthermore, the production of IL-21, which is essential for plasma cell differentiation, tended to be lower in HBsAb− PWH than in non-HIV controls or HBsAb+ PWH.

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Conclusions cTfh cells isolated from HBsAb− PWH may be unable to produce sufficient IL-21 after immune stimulation. This study is the first to suggest that cTfh cells may not function adequately in some PWH. This study highlights the need for large-scale validation of cTfh cell frequency and function in PWH.

Clinical trial number Not applicable.

Keywords Antibodies, Circulating T follicular helper (Tfh) cells, HBV vaccination, People living with HIV (PWH), IL-21

Background

Human immunodeficiency virus (HIV) is an RNA virus belonging to the Retroviridae family that, after infecting CD4+ T cells, is incorporated into the host genome to become a persistently infectious provirus. Although antiretroviral therapy (ART) has improved the prognosis of people living with HIV (PWH) [1], it cannot eliminate the HIV-infected cells themselves, and persistent HIV infection leads to chronic inflammation and various complications [2, 3].

The center of humoral immunity is secondary lymphoid tissues, such as the spleen and lymph nodes. B cells are localized in the follicles of secondary lymphoid tissues. The differentiation of these B cells into plasma cells is maintained by specialized T cells or T follicular helper (Tfh) cells that reside within the follicles. Vaccine-induced antibody production occurs when antigens directly stimulate B cells in follicles, and T cells are stimulated by type 2 dendritic cells that recognize antigens [4–6]. B cells in the follicles become germinal center B (gcB) cells by binding to T cells, which present antigens via dendritic cells. gcB cells undergo positive selection by Tfh cells and follicular dendritic cells and differentiate into antibody-producing plasma cells. Age-related decreases in antibody-producing capacity following vaccination are known to result from the senescence of Tfh cells [7]. Functional analysis of Tfh cells requires secondary lymphoid tissue; however, collecting human Tfh cells via biopsy or other methods is invasive. Some T cells (CD4+CXCR5+ cells), termed circulating Tfh (cTfh) cells and present in the peripheral blood, facilitate immunoglobulin class-switch recombination of B cells and assist in germinal center responses [8, 9]. cTfh cells are clonally related to Tfh cells in secondary lymphoid tissues [10–12]. Because cTfhs can be collected via a minimally invasive blood draw, they have been used to predict Tfh function in humans [10].

Universal hepatitis B virus (HBV) vaccination for 0-year-olds was introduced in Japan in October 2016. The primary HBV vaccines used in Japan are Bimmugen and Heptavax-II, both of which use the HBV small envelope protein as an antigen. The HBV vaccination schedule consists of three doses at 0, 1, and 6 months. These three doses of the HBV vaccine constitute one series. The HBV antibody acquisition rate after one series of HBV vaccinations is 95%, and the protective effect against infection lasts for more than 20 years [13]; however, a certain percentage (5–10%) of the population cannot acquire antibodies [14]. The human leukocyte antigen (HLA) single nucleotide polymorphisms (SNPs) responsible for this phenomenon have been identified and are known to have low reactivity to the antigens used in vaccines [15, 16]. The rate of antibody acquisition with the HBV vaccine in PWH who cannot acquire antibodies (small protein antibody-negative [HBsAb−]) is lower than that in non-HIV controls [14]. These PWH are less likely to acquire antibodies to HBV after HBV vaccination, and even if they do, they have a lower rate of long-term antibody production than non-HIV controls do [17]. However, these phenomena cannot be explained by HLA SNPs alone. HIV infects Tfh cells [18]. These HIV-infected Tfh cells are difficult to eliminate by cellular immunity because they reside in the follicles of secondary lymphoid tissues, a location that is difficult for cellular immunity to reach [19]. Therefore, once HIV infection is established in Tfh cells, the effects of infection may persist for a long period; however, the effects of HIV infection on cTfh cells are unknown.

This study aimed to investigate the frequency and function of Tfh cells in PWH who are HBsAb − after HBV vaccination. We used isolated cTfh cells, the same cell population as Tfh cells in lymphoid tissue, from peripheral blood and compared their functions with those of PWH who became HBV surface protein antibody positive (HBsAb+) after HBV vaccination (HBsAb+ PWH), PWH who remained HBsAb − after vaccination (HBsAb − PWH), and HBsAb+ non-HIV controls (non-HIV controls).

Case presentation

Participants enrolled in this study

PWH without a history of previous HBV vaccination or HBV infection (no positive record of HBs antigen, HBsAb, or HBc antibody or any description of the history of HBV infection within 10 years before vaccination) who received a total of three doses (one series) of the HBV vaccine (Heptavax-II) after November 2021 ($n = 26$) were extracted from the medical records of the Institute of Medical Science, the University of Tokyo (IMSUT) hospital and their data, including age, sex, history of AIDS, HIV-RNA before starting ART, the nadir of CD4+ T cell count, antiretroviral drug regimen, CD4+ T cell count

and HIV viral load at the time of the PBMC sampling, dates of HBV vaccinations, and serial HBsAb titers, were collected (Table 1). PWH recruited for this study maintained undetectable HIV RNA levels in their blood (<50 copies/mL) for over two years through ART. Three PWH who did not test positive for HBsAbs (HBsAbs of < 10 mIU/mL) and five PWH who acquired HBsAbs > 100 mIU/mL were included in the study (Table 1, Fig. 1a). Eight non-HIV controls aged 26 to 48 years (healthy volunteers recruited from our facility) were also enrolled in this study (mean age 37.3 years; Supplementary Table 1 of Additional file 5_2). Non-HIV controls had previously received one series of HBV vaccines and produced sufficient HBsAbs. Non-HIV controls were those who had no fever or other symptoms for 3 days before or after the blood draw. Ethical approval was obtained from the Research Ethics Committee of the University of Tokyo (no. 2023–79-0308). The study complied with the principles of the Declaration of Helsinki. All participants enrolled in this study provided informed consent to participate in the study and for their data to be published.

Three of the 26 people living with HIV (PWH) tested HBsAg– after receiving three doses of the HBV vaccine

Among the 26 PWH who received three doses of the HBV vaccine after November 2021, 22 were tested for HBsAb titers between 30 and 180 d after the third dose of the HBV vaccine (Fig. 1a, Table 1). Among the 22 PWH with postvaccination HBsAb measurements, three had maximum HBsAb titers below 10 mIU/mL (HBsAb–), and 19 had HBsAb titers above 10 mIU/mL (HBsAb+) (Fig. 1b). Seven PWH remained positive for one year after the third HBV vaccination. Two PWH showed a peak between 10 mIU/mL and 100 mIU/mL, and the titers became negative within 1 year after the third HBV vaccination. The other ten PWH reached peaks ranging from 100 to 1000 mIU/mL. Although the follow-up period of the antibody titers in these PWH was less than one year, the titers did not become negative during this period.

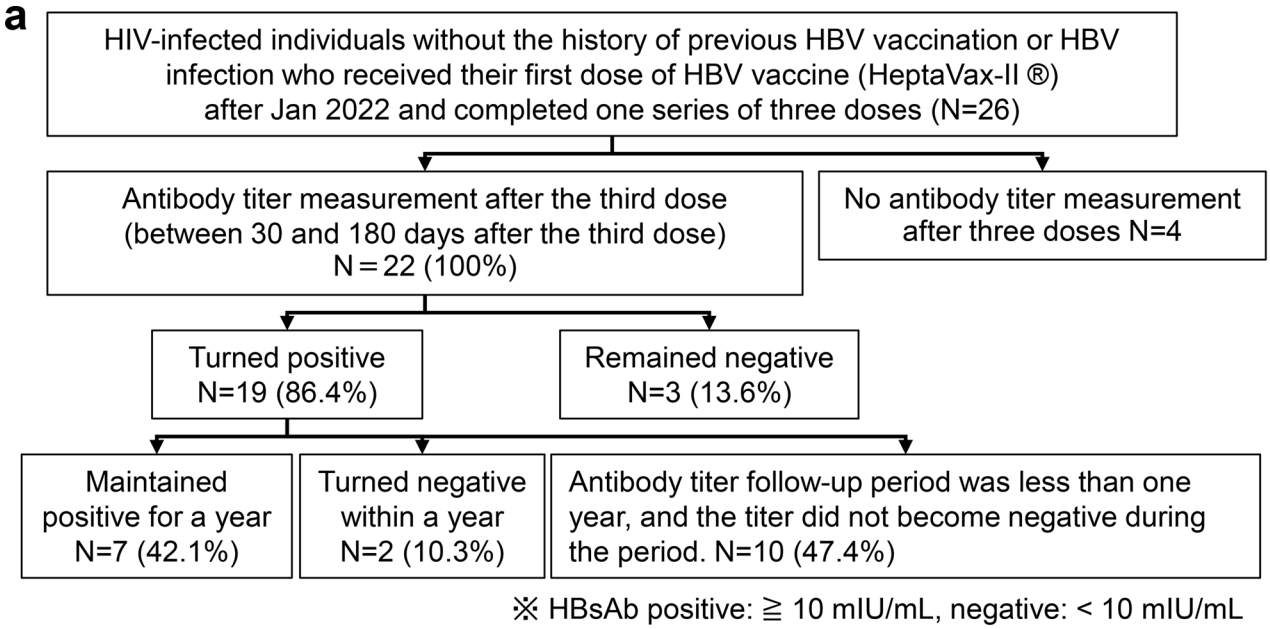
HBsAb– PWH tended to have lower cTfh cells after immune stimulation

To determine the effects of persistent HIV infection on humoral immunity, we compared the frequency and function of medium-treated cTfh cells without immune stimulation (baseline) in three groups: non-HIV controls who had previously received the HBV vaccine and tested positive for HBsAb ($n = 8$), HBsAb+ PWH ($n = 5$), and HBsAb– PWH ($n = 3$). Because the expression pattern of the surface proteins of cTfh cells is CD4+CD45RA–CXCR5+ [20, 21], cTfh cells were immunostained with antibodies against these proteins and examined via flow cytometry (FCM). The gating strategy of the FCM

Table 1 Clinical information for the people living with HIV (PWH) enrolled in this study

Individual No.	Age	Sex	History of AIDS (Yes 1/ No 0)	HIV-RNA before starting ART	Duration of the suppressing regimen (years)	Date of PBMC sampling	Date of the first dose of vaccination	HIV-RNA at the time of PBMC sampling (copies/mL)	CD4+ T cell at the time of PBMC sampling (cells/mL)	Nadir CD4+T cell (cells/mL)	ART regimen	Antibody titer before 1st dose (mIU/mL)	Peak antibody titer after the third dose (mIU/mL)
P1	49	M	0	1400	9	9-Jul-24	14-Jun-22	<50	623	273	BIC/TAF/FTC	0	3.42
P2	55	M	0	15000	8	24-Aug-23	8-Nov-22	<50	925	251	Oral CAB+RPV	0	3.61
P3	69	M	0	93000	10	28-Aug-17	11-Jan-22	<50	355	33	DTG+TAF/FTC	0.18	5.23
P4	45	M	0	19000	7	6-Feb-19	19-Aug-22	<50	579	426	Oral CAB+RPV	4.12	473.77
P5	44	M	1	22000	8	2-Oct-17	16-May-23	<50	609	73	DTG/3TC	0.68	625.09
P6	32	M	0	4100	7	13-Jul-17	2-Nov-21	<50	660	239	DTG+TAF/FTC	0.37	793.12
P7	27	M	0	6100	3	25-Jan-19	9-Aug-22	<50	747	444	DTG/3TC	0.12	1170.86
P8	52	M	0	12000	11	6-Feb-18	16-Aug-22	<50	1189	301	DTG/3TC	0	1964.98

3TC: lamivudine, BIC: bictegravir, CAB: cabotegravir, DTG: dolutegravir, FTC: emtricitabine, RPV: rilpivirine, TAF: tenofovir alafenamide



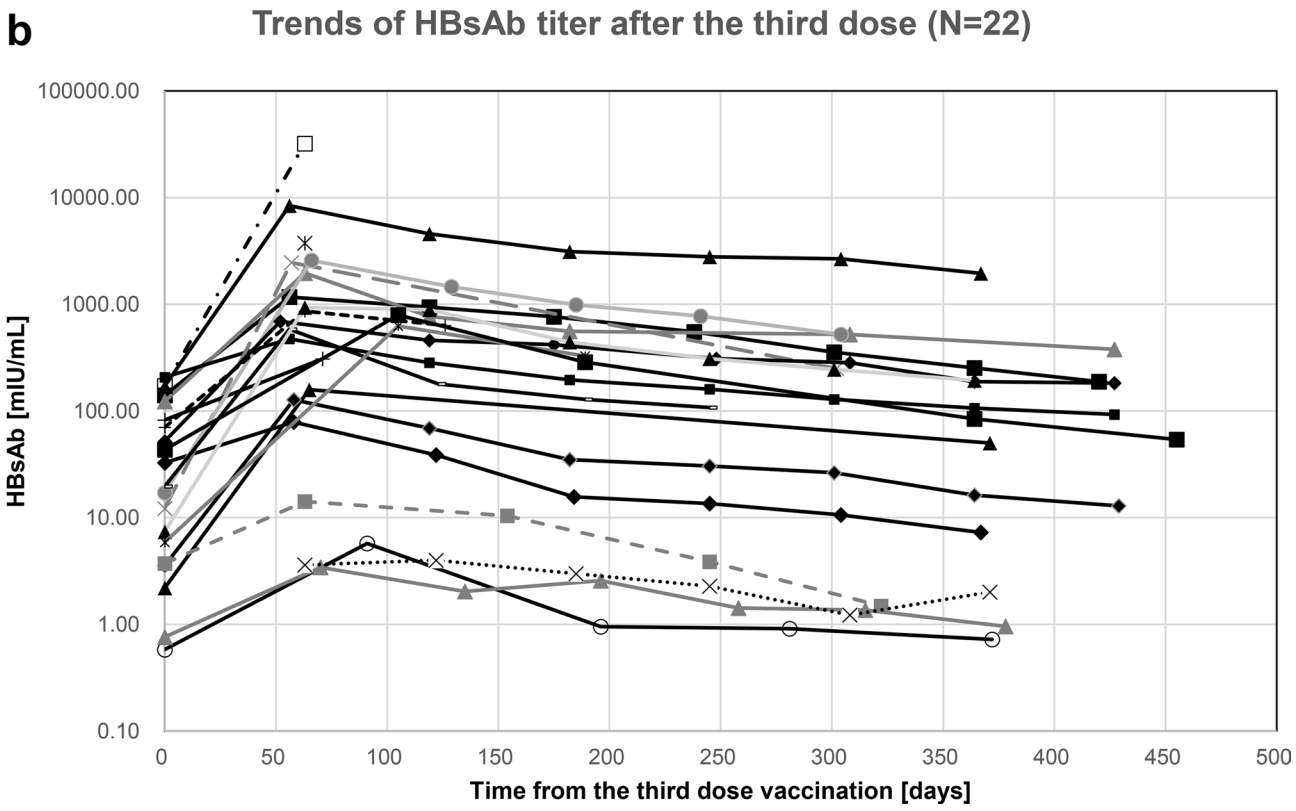


Fig. 1 Overview of the people living with HIV (PWH) enrolled in this study (**a**) Flowchart of PWH vaccinated against HBV. (**b**) Antibody titer after three doses (one series) of the HBV vaccine in PWH

is shown in supplementary Fig. 1 (Additional file 2_2). The methods used for CD4+ T cell isolation, immune stimulation, and FCM analysis are described in supplementary methods (Additional file_6). Figure 2a shows the FCM results for a non-HIV control, HBsAb+ PWH, and HBsAb− PWH. A summary of the FCM results for all PWH in each experimental group is shown in Fig. 2c-h. The results revealed that the frequency of CXCR5+ cTfh cells among CD4+CD45RA − cells was 19.93% in the non-HIV control, 11.51% in the HBsAb+ PWH, and

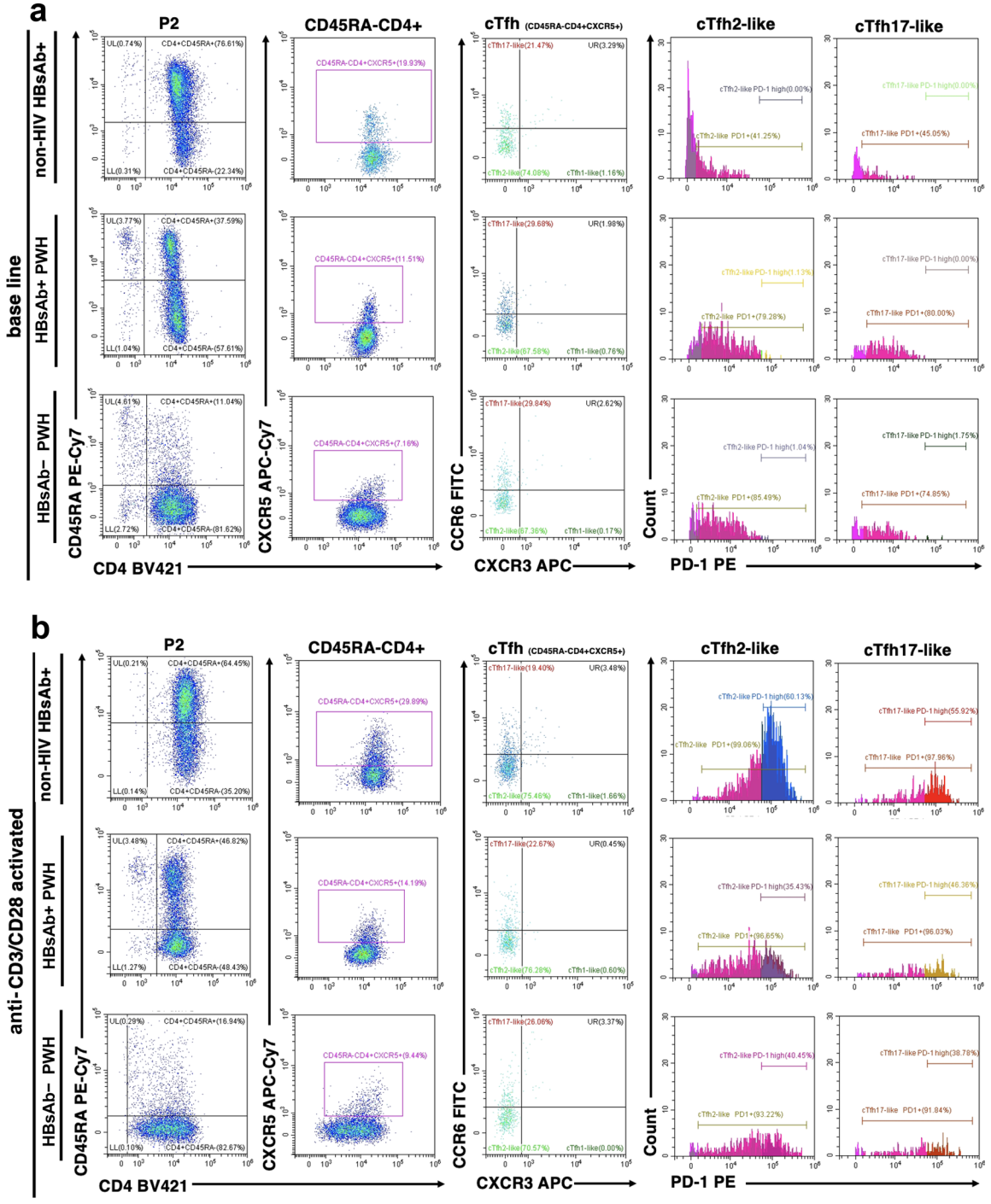


Fig. 2 Frequencies of PD-1+ cTfh17-like cells and PD-1+ cTfh2-like cells in HBsAb− PWH tended to be lower than those in non-HIV controls. (**a**) FCM analysis of CD4+ T cells isolated from a non-HIV control, an HBsAb+ PWH, and an HBsAb− PWH at baseline. cTfh cells: CD4+CD45RA−CXCR5+ cells; cTfh17-like cells: CCR6+CXCR3− cells; cTfh2-like cells: CCR6−CXCR3− cells (**b**) FCM analysis of CD4+ T cells following immune stimulation with anti-CD3/CD28 antibodies (anti-CD3/CD28 activation) in a non-HIV control, an HBsAb+ PWH, and an HBsAb− PWH. (**c**) Frequencies of cTfh cells among CD4+CD45RA− cells in non-HIV controls, HBsAb+ PWH, and HBsAb− PWH. Statistical analysis was performed via the Kruskal–Wallis test with the steel–dwass test. *, p value < 0.05; **, p value < 0.01 (**d**) frequencies of PD-1+ Tfh17-like cells among cTfh cells in non-HIV controls, HBsAb+ PWH, and HBsAb− PWH. (**e**) Frequencies of PD-1+ Tfh2-like cells among cTfh cells in non-HIV controls, HBsAb+ PWH, and HBsAb− PWH. (**f**) Frequencies of cTfh17-like cells highly expressing PD-1 (cTfh17-like PD-1 high) in non-HIV controls, HBsAb+ PWH, and HBsAb− PWH. (**g**) Frequencies of cTfh2-like cells highly expressing PD-1 (cTfh2-like PD-1 high) in non-HIV controls, HBsAb+ PWH, and HBsAb− PWH. (**h**) Frequencies of CD45RA+ CD4+ T cells (naïve T cells) in non-HIV controls, HBsAb+ PWH, and HBsAb− PWH

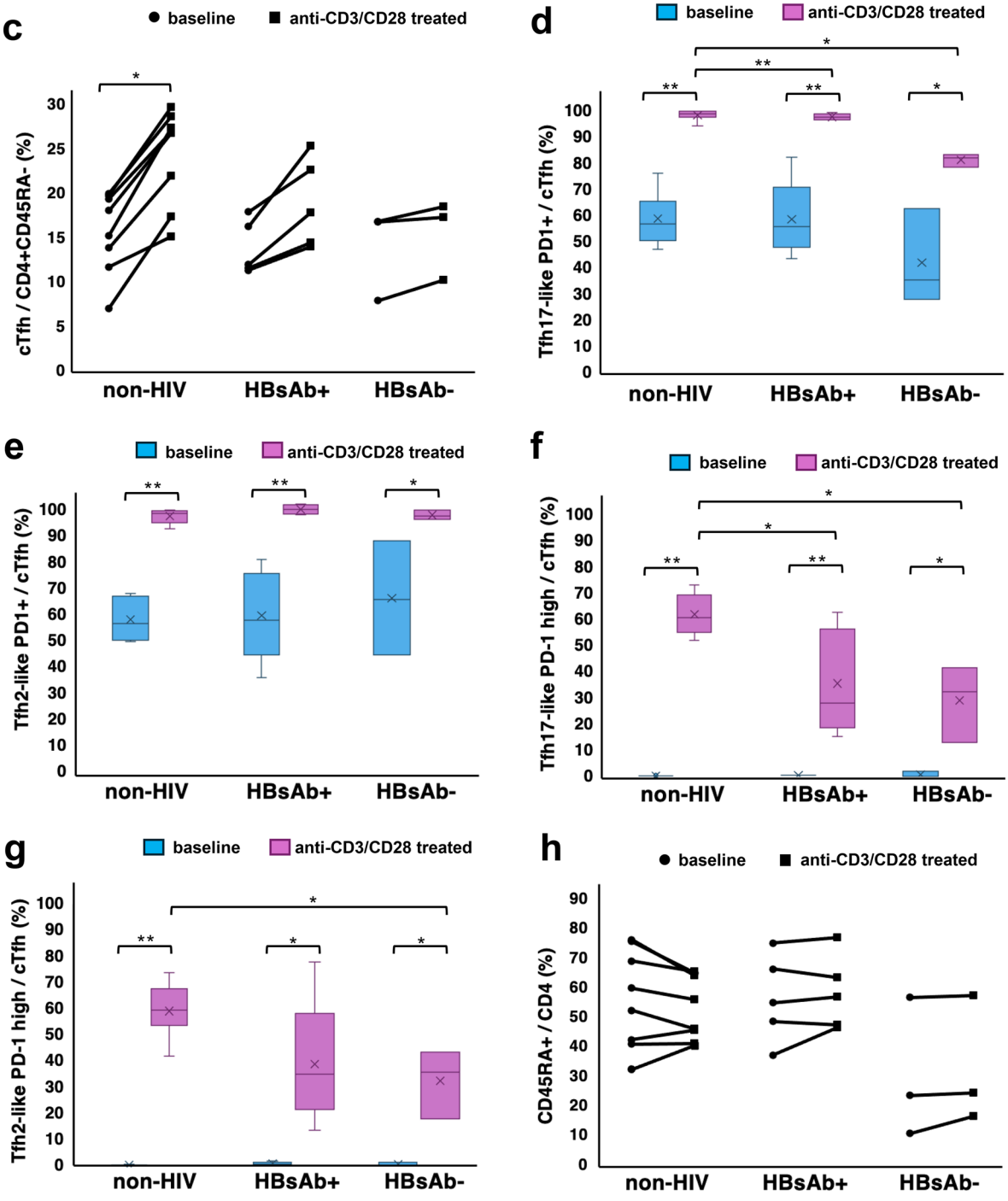


Fig. 2 (continued)

7.16% in the HBsAb– PWH (Fig. 2a). The frequency of cTfh cells in HBsAb – PWH was similar to that in non-HIV controls and HBsAb+ PWH (Fig. 2c). Notably, one of the three PWH tended to have fewer cTfh cells.

After immunostimulation with vaccines, cTfh cells expand and increase their expression of the inhibitory receptor PD-1 [22, 23]. In this study, we compared cTfh cell counts among non-HIV controls, HBsAb+ PWH, and HBsAb – PWH. Fig. 2b shows the FCM results for a non-HIV control, an HBsAb+ PWH, and an HBsAb– PWH. A summary of the FCM results for all individuals in each experimental group is shown in Fig. 2c-h. The results revealed that the frequency of cTfh cells increased after immune stimulation in non-HIV controls and HBsAb+ PWH (Fig. 2b, 2c; p value < 0.05; Supplementary Tables 3 and 4 of Additional file 5_2). In contrast, the frequency of

cTfh cells in HBsAb – PWH remained unchanged after immune stimulation (Supplementary Table 5 of Additional file 5_2). The frequency of cTfh cells after immune stimulation in HBsAb– PWH was significantly lower than that in non-HIV controls (p value < 0.05, Supplementary Table 7 of Additional file 5_2).

The frequencies of PD-1+ cTfh17-like cells and PD-1+ cTfh2-like cells in HBsAb– PWH were lower than those in non-HIV controls

cTfh cells are classified as cTfh17-like (CCR6+CXCR3–), cTfh2-like (CCR6–CXCR3–), or cThf1-like (CCR6–CXCR3+) cells according to their CCR6 and CXCR3 expression patterns [5]. Among these subsets, cTfh17-like and Tfh2-like cells are involved in humoral immunity [24]. In addition, PD-1 is expressed in cTfh cells and regulates cTfh cell function in the germinal center [25]. Therefore, we examined PD-1 expression in cTfh17-like and cTfh2-like cells at baseline. The results revealed that the frequencies of PD-1+ cTfh17-like and PD-1+ cTfh2-like cells did not differ among the three experimental groups (Fig. 2a, d-e). There were no cTfh17-like or cTfh2-like cells that highly expressed PD-1 in the three experimental groups at baseline (Fig. 2a, f-g). The frequencies of cTfh17-like and cTfh2-like cells among the three experimental groups were the same, although there was greater variation in HBsAb – PWH (Supplementary Fig. 2a, b of Additional file 3_2). The frequencies of cTfh17-like and cTfh2-like cells of non-cTfh cells (CD4+CD45RA–CXCR5–) in the three experimental groups are shown in Supplementary Fig. 2c, d (Additional file 3_2).

To examine whether the expression of PD-1 is increased in cTfh17-like and cTfh2-like cells after immune stimulation in non-HIV controls, HBsAb+ PWH, and HBsAb – PWH. In non-HIV controls and HBsAb+ PWH, the frequency of PD-1+ cells among cTfh17-like cells increased after immune stimulation (Fig. 2b, d, p value < 0.05; Supplementary Tables 3 and 4 of Additional file 5_2). In contrast, the frequency of PD-1+ cells among cTfh17-like cells in HBsAb– PWH was lower than that in non-HIV controls and HBsAb+ PWH, although it increased after immune stimulation (p value < 0.05; Supplementary Table 7 of Additional file 5_2). In the case of cTfh2-like cells, the frequency of PD-1+ cTfh2-like cells after immune stimulation was the same across all three experimental groups (Fig. 2b, e, Supplementary Table 5 of Additional file 5_2).

A population of cells expressing PD-1 at high levels that were not detected prior to immune stimulation but appeared after immune stimulation was defined as "high PD-1 expression" cells. In the non-HIV controls, cTfh17-like and cTfh2-like cells, which highly expressed PD-1, appeared at high frequencies after immune stimulation (Fig. 2b, f-g). The frequency of cTfh17-like and cTfh2-like cells with high PD-1 expression was lower in PWH than in non-HIV controls, with HBsAb– PWH exhibiting the lowest frequency (p value < 0.05; Supplementary Table 7 of Additional file 5_2). The results revealed that the frequency of cTfh17-like cells was significantly lower in non-HIV controls (p value < 0.05, Supplementary Table 3 of Additional file 5_2) after immune stimulation but was not different from that before immune stimulation in the PWH groups (Supplementary Fig. 2a of Additional file 3_2). The frequency of cTfh2-like cells in non-HIV controls increased after immune stimulation compared with that before immune stimulation (p value < 0.05, Supplementary Table 3 of Additional file 5_2), did not increase in the PWH groups (Supplementary Fig. 2b of Additional file 3_2), and tended to be lower in HBsAb – PWH than in non-HIV controls (Supplementary Table 7 of Additional file 5_2). The frequency of CD4+CD45RA+ naïve T cells remained unchanged before and after immune stimulation (Fig. 2h).

HBsAb– PWH presented a greater frequency of IL-21+ cTfh cells at baseline and tended to have a lower frequency of IL-21+ cTfh cells after immune stimulation

IL-21 is secreted from differentiated Tfh cells and supports the differentiation and selection of B cells in the germinal centers of secondary lymph nodes [6]. It is important to determine whether the ability of cTfh cells to produce IL-21 is affected by persistent HIV infection to understand why some PWH become HBsAb– after HBV vaccination. In addition, the costimulatory receptor ICOS, which belongs to the CD28 and CTLA-4 cell surface receptor families, is expressed on activated T cells and is involved in Tfh cell differentiation [25]. As the frequency of ICOS+PD-1+ cTfh cells increases after vaccination [22], the differentiation potential of cTfh cells can be determined by examining the frequency of ICOS+PD-1+ cTfh cells after immune stimulation. Therefore, we examined the expression of ICOS and PD-1 and the ability of cTfh cells to produce IL-21 before (baseline) and after (anti-CD3/CD28-activated) immune stimulation via FCM. Figure 3a and b show the FCM results for a non-HIV control, an HBsAb+ PWH, and an HBsAb– PWH, and a summary of all the FCM results is shown in Fig. 3c and d. The gating strategy of the FCM is shown in supplementary Fig. 3 (Additional file 4_2). The frequency of ICOS+ PD-1+ cTfh cells at baseline was the same across all three experimental groups (Fig. 3a–b, c). The frequency of ICOS+PD-1+ cTfh cells increased significantly after immune stimulation, with nearly all cTfh cells becoming ICOS+PD-1+. The frequency of IL-21+ cTfh cells at baseline was low in non-HIV controls and HBsAb+ PWH, whereas the frequency of IL-21+ cTfh cells was significantly greater in HBsAb– PWH than in non-HIV controls or HBsAb+ PWH (Fig. 3a, d, p

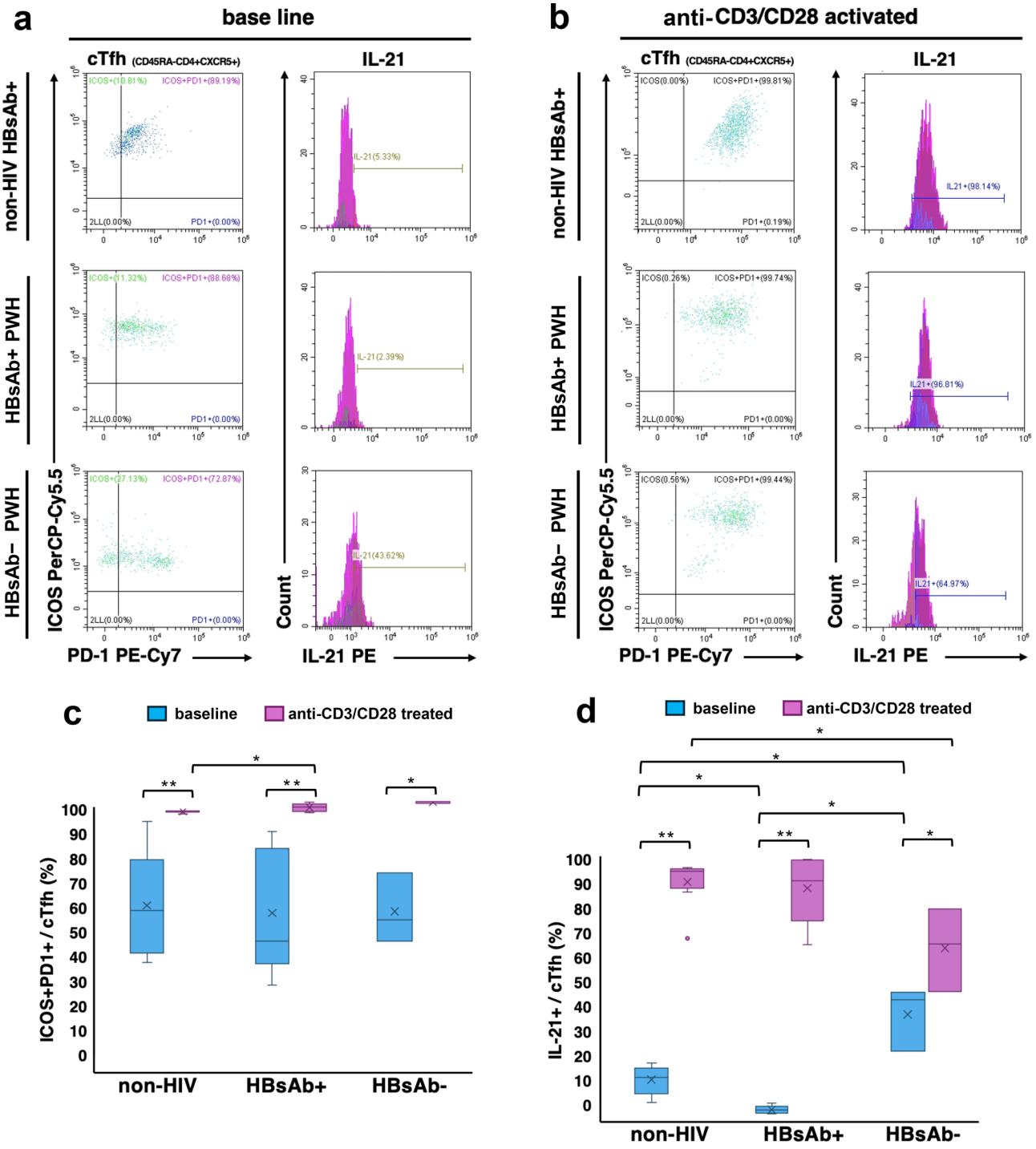


Fig. 3 HBsAb− PWH presented a greater frequency of IL-21+ cTfh cells at baseline and tended to have lower IL-21+ cTfh cells than non-HIV controls and HBsAb+ PWH. (**a**) FCM analysis of cTfh cells isolated from a non-HIV control, an HBsAb+ PWH, and an HBsAb− PWH (baseline). (**b**) FCM analysis of cTfh cells treated with anti-CD3/CD28 antibody in a non-HIV control, HBsAb+ PWH, and HBsAb− PWH (anti-CD3/CD28 activated). (**c**) Frequencies of ICOS+PD1+ cTfh cells in non-HIV controls, HBsAb+ PWH, and HBsAb− PWH. Statistical analysis was performed via the Kruskal–Wallis test with the steel–dwass test. *, *p* value < 0.05; **, *p* value < 0.01 (**d**) Frequencies of IL-21+ cTfh cells in non-HIV controls, HBsAb+ PWH, and HBsAb− PWH

value < 0.05; Supplementary Table 6 of Additional file 5_2). After immune stimulation, the frequency of IL-21 increased significantly, and almost all cTfh cells became IL-21+ in non-HIV controls and HBsAb+ PWH (Fig. 3b, d, *p* value < 0.05; Supplementary Tables 3 and 4 of Additional file 5_2). In contrast, although the frequency of IL-21+ cTfh cells increased in HBsAb − PWH, the frequency was significantly lower than that in non-HIV

controls (p value < 0.05, Supplementary Table 7 of Additional file 5_2).

Discussion and conclusions

The antibody positivity rate of three doses (one series) of the HBV vaccine was lower in PWH than in non-HIV controls [17]. The main players in humoral immunity are B cells, which reside in the germinal centers of secondary lymphoid tissues and require Tfh cells. HIV also infects Tfh cells [18], but Tfh cells infected with HIV are difficult to eliminate because they reside in the follicles of secondary lymphoid tissues, a location that is out of reach of cellular immunity [19]. If the frequency of Tfh cells is reduced or their ability to secrete cytokines, such as IL-21, is impaired, they may not adequately assist B cells in plasma cell differentiation.

A certain percentage (5–10%) of non-HIV controls fail to acquire antibodies from the HB vaccine, and causative HLA SNPs have been identified [15, 16]. However, even if they have SNPs in their HLA gene that reduce their reactivity to the HBV vaccine, their cTfh frequency is the same as that of those who have acquired sufficient antibodies from the HBV vaccine [26]. Although their reactivity to HBV vaccine antigens is reduced by SNPs in HLA, their humoral immunity to other antigens is normal and not caused by Tfh cell dysfunction. However, the results of this study indicated that in HBsAb– PWH, dysfunction of cTfh cells may be the cause. The frequency of cTfh cells in HBsAb – PWH was similar to that in non-HIV controls and HBsAb+ PWH; however, a tendency for the cTfh cell frequency to increase less readily after immune stimulation was observed. Furthermore, almost all cTfh cells isolated from non-HIV controls and HBsAb+ PWH became IL-21+ after immune stimulation, whereas HBsAb– PWH tended toward a lower frequency of cTfh cells becoming IL-21+ after immune stimulation than non-HIV controls or HBsAb+ PWH despite the higher frequency of IL-21+ cTfh cells at baseline. The tendency toward insufficient expansion and inadequate IL-21 production of CXCR5-positive cTfh cells after immune stimulation in HBsAb– PWH is consistent with the findings of a previous study on PWH with a low response to the influenza vaccine [21]. These results suggest that a subset of cTfh cells in HBsAb– PWH may be unresponsive to immune stimulation or may include a cell population that has become exhausted and hyporesponsive to immune stimulation.

Hyporesponsiveness to the HBV vaccine in PWH may be more closely related to age than to HLA SNPs in non-HIV controls. Aging decreases the frequency of cTfh cells after immune stimulation [27]. In this study, the frequency of ICOS+PD-1+ cTfh cells before and after immune stimulation did not differ among the three comparison groups; however, the frequencies of high PD-1-expressing cTfh17-like and cTfh2-like cells after immune stimulation were lower in the PWH groups than in the non-HIV controls. In particular, the frequency of PD-1-expressing cTfh17-like and cTfh2-like cells was lower in HBsAb– PWH than in HBsAb+ PWH. The characteristics of cTfh cells in these HBsAb– PWH were similar to the age-related changes in cTfh cells. The characteristics of cTfh cells observed in HBsAb – PWH are similar to those of age-related changes in cTfh cells [3, 28]. However, it cannot be ruled out that the cTfh cells of HBsAb– PWH may differ from those of aging individuals because the cTfh cells of aging individuals without HIV maintain IL-21 production after immune stimulation [23, 29, 30]. To explore the differences in immune senescence between the cTfh cells of non-HIV controls and HBsAb – PWH, the expression of genetic markers of immune senescence in the cTfh cells of HBsAb – PWH should be examined in the future.

HBV infection occurs through vertical transmission during mother–child transmission as well as horizontal transmission as a sexually transmitted infection. PWH represent an extremely high-risk group for HBV infection, making protection against infection through the HBV vaccine critically important. The HBV vaccine targets a small protein of HBV, and following vaccination, antibodies targeting this small protein (HBsAb) are produced. This HBsAb functions to protect against HBV infection, but HBV infection can still occur even after vaccination. One cause is HBV mutation. Mutations in the small protein of HBV can result in immune escape mutations that evade HBsAb [31, 32]. Another reason is the failure to produce antibodies despite vaccination. In this study, three PWH failed to produce HBsAb after HBV vaccination and tended toward low IL-21 production by cTfh cells. Investigating the effects of HIV infection on Tfh cells will be an important challenge for future HBV vaccination strategies in PWH.

Our study had several limitations. Owing to the very limited number of PWH meeting the eligibility criteria for recruitment in this study, the cohort of PWH included in this research became very small. Furthermore, this study was unable to examine the correlation between all clinical information and the frequency or function of cTfh cells in PWH. Therefore, the insights gained from the findings of this study are very limited. Large-scale studies examining cTfh cell function in PWH are scarce. It would be necessary to eliminate restrictions such as HBV vaccine reactivity, broaden the study population to recruit more PWH, and verify the frequency and function of cTfh cells alongside extensive clinical information, including ART. Second, the HLA SNPs in HBsAb+ PWH studied here remain unknown. The presence of HLA SNPs results in individuals who cannot produce antibodies even after HBV vaccination, significantly reducing

their reactivity to HBV antigens. These SNPs reduce reactivity only to the antigen used in HBV vaccines and do not affect reactivity to other antigens. To mitigate the influence of SNPs, this study employed anti-CD3/CD28 antibodies instead of HBV antigens for immune stimulation; however, this approach does not completely eliminate the potential impact of SNPs. Future research should investigate the presence of SNPs in the HLA region of HBsAb− PWH via whole-genome sequencing.

This study investigated the frequency and function of cTfh cells isolated from HBsAb− PWH in a very small cohort of PWH who received three doses of HBV vaccine. Compared with non-HIV control and HBsAb+ PWH cells, cTfh cells isolated from HBsAb− PWH presented a greater frequency of IL-21+ cTfh cells at baseline, whereas after immune stimulation, the frequency of IL-21+ cTfh cells tended to be lower than that in other experimental groups. Although the very small sample size of this study limits the conclusions that can be drawn from the results, this study suggests that larger-scale, detailed investigations are needed regarding the frequency and function of cTfh cells in PWH.

Abbreviations

cTfh cells	circulating follicular helper T cells
HIV	Human immunodeficiency virus
PWH	People living with HIV
HBV	Hepatitis B virus
HBsAb+	HBV small protein antibody-positive
HBsAb−	HBV small protein antibody-negative
HLA	Human leukocyte antigen
SNP	Single−nucleotide polymorphism
IL−21	Interleukin−21
PD−1	Programmed cell death−1
PBMC	Peripheral blood mononuclear cell
PCR	Polymerase chain reaction
FCM	Flow cytometry
BFA	Brefeldin

Supplementary information

The online version contains supplementary material available at https://doi.org/10.1186/s12879-026-13027-w.

Supplementary Material 1

Supplementary Material 2

Supplementary Material 3

Supplementary Material 4

Supplementary Material 5

Supplementary Material 6

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Author contributions
A.S. performed all the experiments, contributed to data acquisition, and wrote and revised the manuscript. Y.K. collected the clinical information of HBV-vaccinated people living with HIV (PWH). K.Ari. contributed to the statistical analysis. H.Y. designed the study and obtained a grant to conduct the study. K.Ara. contributed to the data acquisition. M.K. contributed to the recruitment of PWH. M.S., Y.K., F.N., and E.A. collected peripheral blood samples. All the authors reviewed and approved the final version of the manuscript.

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Data availability
The FCM data that support the findings of this study are available in the article and supplementary material.

Declaration

Ethical approval and consent to participate
Ethical approval was obtained from the Research Ethics Committee of the University of Tokyo (no. 2023-79-0308). All participants enrolled in this study provided informed consent to participate in the study and for their data to be published.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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