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Letter to the Editor

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Generation of HBsAg-reactive T- and B-cells following HBV vaccination in serological non-responders under hemodialysis treatment

Hemodialysis patients (HDP) are at high risk of hepatitis B virus (HBV) infection. Hence, stringent precautions including HBV vaccination are recommended for seronegative HDP [1]. Although a four times higher vaccination dose is used in HDP compared to healthy individuals, only 50–60% seroconvert [2]. Reasons for the low seroconversion rate remain unknown.

This study characterized HBV vaccination-induced hepatitis B surface antigen (HBsAg)-reactive T and B cells in HDP. The success of HBV vaccination is evaluated 4–6 weeks after vaccination. Anti-HBsAg antibody titers above 10 IU/L are considered to be responsive against HBV infection [1]. Here, seven out of 17 HDP were identified as non-responders, since they failed developing anti-HBsAg titers above 10 IU/L (Supporting Information Table 1). To determine whether transient anti-HBsAg antibodies peaked and waned before day 42 post vaccination, we measured the anti-HBsAg titers in serum samples obtained weekly from all study participants post vaccination until day 28 (Fig. 1A). HDP non-responders did not develop anti-HBsAg titers above 10 IU/L during the observation period. Most HDP responders and healthy controls (HC) showed increased anti-HBsAg titers seven or 14 days after vaccination (Fig. 1A). A general malfunction such as agammaglobulinemia, which could underlie impaired anti-HBsAg titers, [3] was excluded, since total Ig serum levels were within normal ranges for all patients (Supporting Information Fig. 1) [4]. Before vaccination, numbers of circulating B/T cells, and monocytes were similar in all groups, however HDP non-responder had significantly lower numbers of $CD27^{pos}$ B cells (Supporting Information Fig. 2A–D).

The HBsAg-specific B cell response was analyzed by flow cytometry using Cy5- or FITC-conjugated HBsAg. We focused on $CD3^{neg}CD14^{neg}CD19^{pos}CD20^{pos}$ B cells, $CD27^{neg}$ (mainly) mature naïve B cells, $CD27^{pos}$ pre- and post-switched memory B cells, and $CD27^{high}$ antibody-secreting cells (ASC; Supporting Information Fig. 2E) [5, 6]. Before HBV vaccination, we detected similar numbers of HBsAg-binding $CD27^{neg}$ and $CD27^{pos}$ B cells in HDP-responders and -non-responders (Fig. 1B-D). Numbers of $CD27^{neg}$ and $CD27^{pos}$ HBsAg-specific B cells peaked on day 14 after vaccination with similar kinetics for all groups (Fig. 1B-D). For HC, an increase of HBsAg-specific $CD27^{high}$ ASC occurred 7 days after HBV vaccination agreeing with kinetics observed for other booster vaccinations [7]. HBsAg-specific $CD27^{high}$ ASC were detected in HDP-responder and -non-responder delayed and with lower levels, but without significant differences (Fig. 1E).

Overall, the kinetics of vaccination-induced HBsAg-specific B cell response does not explain the lack of seroconversion in HDP non-responders. We could not detect a correlation between antibody production levels and HBsAg-specific B cell subsets (Supporting Information Fig. 3A-C).

Next, we hypothesized that the inability of HDP non-responders to develop anti-HBsAg antibodies could be caused by insufficient generation of HBsAg-reactive $CD4^{pos}$ T cells. Thus, we compared vaccination-induced HBsAg-reactive $CD4^{pos}CD154^{pos}$ T cell responses between HDP-responders and -non-responders (Supporting Information Fig. 4). Before vaccination, a low frequency of HBsAg-reactive $CD4^{pos}$ T cells was detected in most patients without significant differences between HDP groups (Fig. 2A, B). Frequencies of vaccine-induced HBsAg-reactive $CD4^{pos}$ T cells followed similar kinetics in HDP-responders and -non-responders (Fig. 2B). The highest HBsAg-reactive $CD4^{pos}$ T cell levels were observed between days 14 and 21 without significant differences between the two HDP groups (Fig. 2B). Antibody levels and the HBsAg-reactive $CD4^{pos}$ T cells did not correlate (Supporting Information Fig. 3D). The antigen presentation capacity was similar between the HDP groups, since HBsAg-protein and -peptides induced a similar T cells response (Supporting Information Fig. 5A).

The T cell functionality assessment revealed lower frequencies of IFNγ- and TNFα-producing HBsAg-reactive T cells in HDP compared to HC, without significant difference between both HDP

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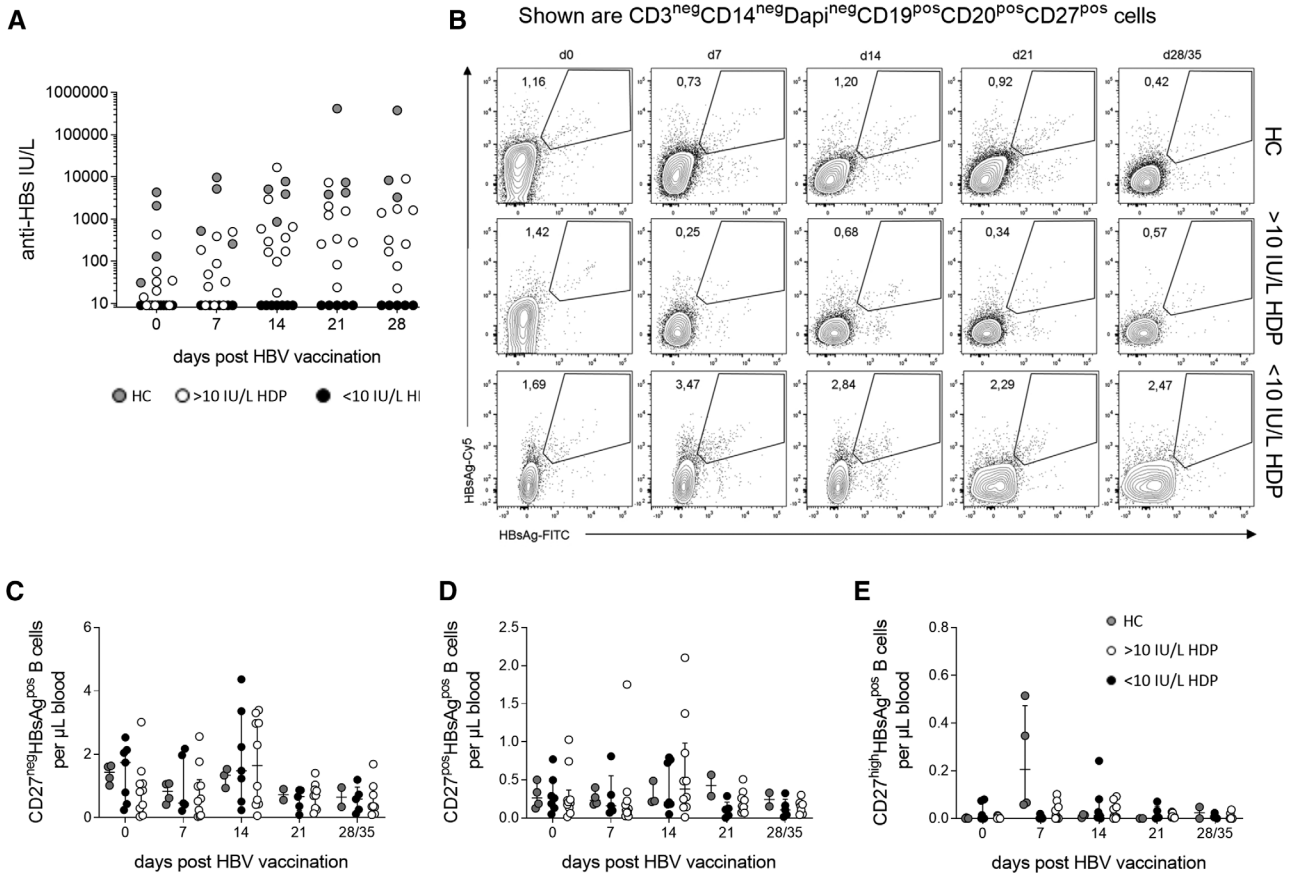

Figure 1. HBsAg-specific B cell response after HBV booster vaccination. (A) Serum samples from HC, HDP responders, and HDP non-responders analyzed by an electro chemiluminescence immuno-assay for anti-HBsAg antibody titers prior to and weekly after HBV vaccination. To avoid batch effects and to ensure independent experiments, donors were vaccinated in five groups of three to five study participants over a period of six month always including responders and non-responders. HC, ((grey) n = 4 for day 0–21; n = 3 for day 28). HDP responders, ((white) n = 10 for day 0–14, n = 9 for day 21 and 28). HDP non-responders, ((black) n = 7 for day 0 and 14; n = 5 for day 7, 21, and 28). (B) Representative flow cytometry dot plots showing the kinetics of HBsAg-specific B cells CD27^{pos} B cells before (d0) and weekly after HBV booster vaccination for HC (top), HDP responder (middle) and HDP non-responder (bottom). (C) Absolute counts of CD27^{neg} (D) of CD27^{pos} (E) of CD27^{high} HBsAg-specific B cells after HBV booster vaccination. HC, ((grey) n = 4 for day 0 and 7; n = 3 for day 14, n = 2 for day 21 and day28/35). HDP responders, ((white) n = 10 for day 0, 7, and 14; n = 9 for day 21 and n = 8 for day 28/35). HDP non-responders, ((black) n = 7 for day 0 and day 14; n = 5 for day 7, 21 and 28/35). Two-way Anova tests with a Tukey posttest for multiple comparison were performed. Median with interquartile range is shown. Data are combined from 5 independent experiments.

groups (Fig. 2C-E). The frequencies of IL-2 and granzyme-B-producing HBsAg-reactive T cells were similar in all groups (Supporting Information Fig. 5B, C). CCR7^{neg}CD45RA^{neg} T cells include effector memory and follicular helper T cells (TFH). Expansion of this T cell population was observed in HC, whereas in HDP, frequencies of CCR7^{neg}CD45RA^{neg} HBsAg-reactive T cells remained constant throughout the observation period (Supporting Information Fig. 6).

This functional evaluation demonstrates that both HDP groups develop functionally comparable HBsAg-reactive T cells.

Our study highlights that HDP can generate HBsAg-reactive T and B cells regardless of their post vaccination serological response prompting more intensive investigations of vaccination-induced immune responses in HDP including reconsideration of vaccination strategies and efficacy assessment. Future studies should consider analyzing further subsets of HBsAg-reactive T cells, including TFH cells that support B cells by providing IL-21 [8, 9]. ELISPOTs could elucidate whether the differences are due to antibody quantities produced by individual cells. To elaborate whether the observed effects are specific for HBV vaccinations, studies using other vaccines such as against influenza should be performed in HDP [10].

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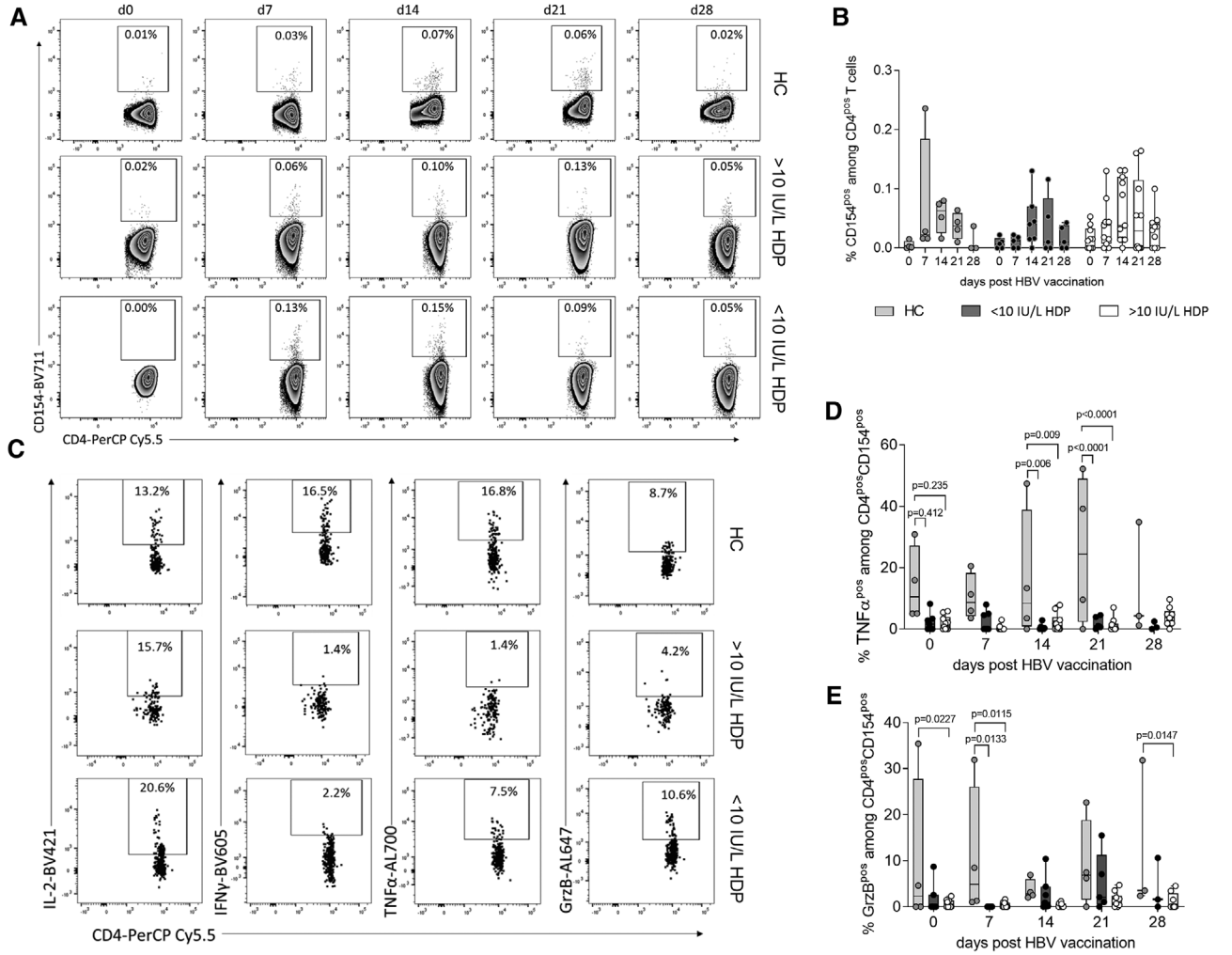

Figure 2. Functional CD4^{pos} HBsAg-reactive T cells developed in all participants groups. (A) Representative flow cytometry dot plots showing the development of HBsAg-reactive T cells over 28 days from HC (top), HDP responders (middle) and HDP non-responders (bottom). (B) Frequencies of HBsAg-reactive CD4^{pos}CD154^{pos} T cells comparing the kinetic for each participant group. Experimental setup and donor numbers are as described for figure 1 A. (C) Representative flow cytometry dot plots illustrating the expression of IL-2, IFN-γ, TNF-α, and GrzB in the CD4^{pos}CD154^{pos} HBsAg-reactive T cells of HC (top), HDP responder (middle) and HDP non-responder (bottom) on day 7 post vaccination. (D, E) Average TNF-α (D), and GrzB (E) expression in the CD4^{pos}CD154^{pos} HBsAg-reactive T cells of all participant groups as described for (B) measured weekly post HBV vaccination until day 28. Boxes represent the 25th, 50th, and 75th percentile; whiskers range from the minimum to the maximum value. A Two-way ANOVA with a Tukey multiple comparison post-test was used to statistically compare cell frequencies between groups. Data are combined from five independent experiments.

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