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Hepatitis B virus-specific effector CD8$^+$ T cells are an important determinant of disease prognosis: A meta-analysis

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ABSTRACT

Background: Hepatitis B virus (HBV)-specific effector CD8$^+$ T cells are critical for viral clearance. To determine the effects of HBV-specific effector CD8$^+$ T cells on HBV infection, we performed a meta-analysis of the available literature.

Methods: Electronic database searches identified appropriately designed studies that detected specific CD8$^+$ T cells in HBV-infected patients. Our main endpoints were the course of infection, seroconversion of HBV "e" antigen (HBeAg), the level of HBVDNA, and alanine aminotransferase (ALT) activity. We used a fixed/random model for analysis, according to the results of a heterogeneity test (P value of Q-squared, I^2).

Results: Our searches found five eligible articles. Pooled estimation of the reported results showed that levels of specific CD8$^+$ T cells were significantly higher in patients with acute hepatitis B than in patients with chronic hepatitis B (odds ratio [OR] = 76.30, 95% confidence interval [CI]: 15.37–378.70). With respect to chronic hepatitis B, patients with $<10^7$ copies/ml HBVDNA had higher levels of specific CD8$^+$ T cells relative to patients with $>10^7$ copies/ml HBVDNA, but the difference had no statistics significance (OR: 3.89, 95% CI: 0.71–21.33). Patients with negative HBeAg or positive anti-HBeAg antibody (anti-HBe) results had significantly higher levels of specific CD8$^+$ T cells versus patients with positive HBeAg results (OR: 5.82, 95% CI: 1.41–24.13). There were no significant associations between the levels of specific CD8$^+$ T cells and serum ALT activity (OR = 0.86, 95% CI: 0.01–74.15).

Conclusion: HBV-specific effector CD8$^+$ T cells influence the disease activity in HBV-infected patients in various ways and determine prognosis by eliminating the virus. Therefore, efforts of studying HBV-specific effector CD8$^+$ T cells focused vaccine are potentially needed.

1. Introduction

Chronic hepatitis B virus (HBV) infection (CHB) is a public health problem worldwide, with 240 million chronic carriers. Although prophylactic vaccines exist, there is no available treatment to cure chronically infected individuals, who are at a high risk of liver cirrhosis and hepatocellular carcinoma (HCC) [1,2]. Current treatment options available for CHB include pegylated interferon alpha2a and nucleos(t)ide analogues [3]; however, both of these options can not thoroughly eliminate the virus. In addition, they are not curative measures because they do not target covalently closed circular (ccc) DNA directly [4]. There have been many innovative treatment concepts regarding CHB, and multiple research efforts have been made in this direction, which include direct-acting antivirals and host-targeting agents [5,6]. Direct-acting antivirals include agents targeting HBV capsids [7,8], inhibitors of cccDNA formation [9], DNA-cleaving enzymes [10,11], and antisense oligonucleotides [12]. Host-targeting agents include agents targeting sodium taurocholate cotransporting polypeptide (NTCP) [13], lymphotoxin-β receptor agonists [14], programmed cell death ligand 1 (PD-L1) blockers [15,16], and a therapeutic vaccine [17]. Nonetheless, these strategies have been under development, and their effects are uncertain. Therefore, it is urgently necessary to search for novel strategies to cure CHB or achieve long-term suppression of the virus.

To search for novel strategies, the mechanisms of interactions between the virus and host immune system should be further characterized. While a segment of patients develop CHB, others get rid of HBV infection without any clinical consequences; an observation that has confused researchers for many years [18]. It had been thought that the presence of functional HBV-specific T cells and HBV-specific antibody–producing B cells determines the prognosis and outcome of HBV infection [19].

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Because of secretion of antiviral cytokines and killing of infected cells, specific $CD8^+$ T cells are major contributors to tissue immunopathology as well as to viral clearance [20]. Antigen-specific effector $CD8^+$ T cells are crucial for controlling CHB [21], and persistent infection by HBV is characterized by virus-specific and global T-cell dysfunction, regulated by multiple mechanisms [22]. Furthermore, in murine models, a low HBV-specific T-cell response correlates with high regulatory-T-cell (Treg) numbers, which contribute to persistent HBV infection [23]. In addition, some researchers have used T-cell therapy for CHB and HBV-induced HCC [24]. To sum up, virus-specific T cells may play a critical role in the prognosis and outcome of CHB. Nevertheless, during some acute viral infections, in addition to producing proinflammatory cytokines, specific $CD8^+$ T cells secrete the regulatory cytokine IL-10 [25–28], which is generally thought to limit tissue damage. Therefore, the function of virus-specific effector $CD8^+$ T cells in CHB is a controversial topic.

Many therapeutic vaccines and immunotherapeutic strategies against HBV are being developed or are currently at the stage of clinical studies [29]. Most of these strategies focus on how to induce an adaptive immune response, especially that involving specific T cells. Therefore, determination of the effect of HBV-specific effector $CD8^+$ T cells on the control of HBV infection is crucial. The frequency and function of Tregs in CHB have been reported in a meta-analysis [30]; however, the effects of virus-specific effector $CD8^+$ T cells on the prognosis of hepatitis B infection, especially on the course of the disease, seroconversion of HBV e antigens (HBeAg) to antibodies, levels of HBVDNA, and alanine aminotransferase (ALT) activity remain controversial. Therefore, the purpose of this study is to evaluate available evidence on the effects of specific $CD8^+$ T cells on HBV infection via a systematic review of the literature and meta-analysis of the reported results.

2. Materials and methods

2.1. The search strategy

An electronic database search was conducted in PubMed, Embase, Cochrane, and OVID using the following medical subject headings (MeSH) terms: "hepatitis B virus,""cytotoxic T lymphocytes," and "CD8 positive T lymphocytes." The complete searches are shown in Tables 1–3. A manual search was also performed, in the reference lists of key articles. No restrictions were set in terms of study design, language, sample size, or outcome of each study. Additionally, names of authors or journals did not influence our decisions to include or excludean article. All articles were identi-

Table 1
The complete searches in PubMed.

Step no.	Search terms
1	Hepatitis B virus [MeSH] OR B virus, Hepatitis OR Hepatitis B viruses OR viruses, Hepatitis B OR Hepatitis Virus, Homologous Serum OR Dane Particle OR Particle, Dane
2	Cytotoxic T lymphocytes [MeSH] OR Cytotoxic T-Lymphocyte OR T Lymphocytes, Cytotoxic OR T-Lymphocyte, Cytotoxic OR Cell-Mediated Lympholytic Cells OR Cell Mediated Lympholytic Cells OR Cell, Cell-Mediated Lympholytic OR Cell-Mediated Lympholytic Cell OR Cells, Cell-Mediated Lympholytic OR Lympholytic Cell, Cell-Mediated OR Lympholytic Cells, Cell-Mediated OR Cytotoxic T-Lymphocytes OR Cytotoxic T Lymphocytes OR TC2 Cells OR Cell, TC2 OR Cells, TC2 OR TC2 Cell OR TC1 Cells OR Cell, TC1 OR Cells, TC1 OR TC1 Cell
3	CD8 Positive T Lymphocytes [MeSH] OR CD8-Positive T-Lymphocyte OR T-Lymphocyte, CD8-Positive OR T-Lymphocytes, CD8-Positive OR T8 Lymphocytes OR Lymphocyte, T8 OR Lymphocytes, T8 OR T8 Lymphocyte OR CD8-Positive Lymphocytes OR CD8 Positive Lymphocytes OR CD8-Positive Lymphocyte OR Lymphocyte, CD8-Positive OR Lymphocytes, CD8-Positive OR T8 Cells OR Cell, T8 OR Cells, T8 OR T8 Cell OR Suppressor T-Lymphocytes, CD8-Positive OR CD8-Positive Suppressor T-Lymphocyte OR CD8-Positive Suppressor T-Lymphocytes OR Suppressor T Lymphocytes, CD8 Positive OR Suppressor T-Lymphocyte, CD8-Positive OR T-Lymphocyte, CD8-Positive Suppressor OR T-Lymphocytes, CD8-Positive Suppressor OR Suppressor T-Cells, CD8-Positive OR CD8-Positive Suppressor T-Cell OR CD8-Positive Suppressor T-Cells OR Suppressor T Cells, CD8 Positive OR Suppressor T-Cell, CD8-Positive OR T-Cell, CD8-Positive Suppressor OR T-Cells, CD8-Positive Suppressor
4	#2 OR #3
5	#1 AND #4

Table 2
The complete searches in Embase and Cochrane.

Search strategy
#1 Hepatitis B virus [MeSH]; #2 B virus, Hepatitis; #3 Hepatitis B viruses; #4 viruses, Hepatitis B; #5 Hepatitis Virus, Homologous Serum; #6 Dane Particle; #7 Particle, Dane
#8 #1 or #2 or #3 or #4 or #5 or #6 or #7
#9 T-Lymphocytes, Cytotoxic [MeSH]; #10 Cytotoxic T-Lymphocyte; #11 T Lymphocytes, Cytotoxic; #12 T-Lymphocyte, Cytotoxic; #13 Cell-Mediated Lympholytic Cells; #14 Cell Mediated Lympholytic Cells; #15 Cell, Cell-Mediated Lympholytic; #16 Cell-Mediated Lympholytic Cell; #17 Cells, Cell-Mediated Lympholytic; #18 Lympholytic Cell, Cell-Mediated; #19 Lympholytic Cells, Cell-Mediated; #20 Cytotoxic T-Lymphocytes; #21 Cytotoxic T Lymphocytes; #22 TC2 Cells; #23 Cell, TC2; #24 Cells, TC2; #25 TC2 Cell; #26 TC1 Cells; #27 Cell, TC1; #28 Cells, TC1; #29 TC1 Cell
#30 #9 or #10 or #11 or #12 or #13 or #14 or #15 or #16 or #17 or #18 or #19 or #20 or #21 or #22 or #23 or #24 or #25 or #26 or #27 or #28 or #29
#31 CD8-Positive T-Lymphocytes [MeSH]; #32 CD8-Positive T-Lymphocyte; #33 T-Lymphocyte, CD8-Positive; #34 T-Lymphocytes, CD8-Positive; #35 T8 Lymphocytes; #36 Lymphocyte, T8; #37 Lymphocytes, T8; #38 T8 Lymphocyte; #39 CD8-Positive Lymphocytes; #40 CD8 Positive Lymphocytes; #41 CD8-Positive Lymphocyte; #42 Lymphocyte, CD8-Positive; #43 Lymphocytes, CD8-Positive; #44 T8 Cells; #45 Cell, T8; #46 Cells, T8; #47 T8 Cell; #48 Suppressor T-Lymphocytes, CD8-Positive; #49 CD8-Positive Suppressor T-Lymphocyte; #50 CD8-Positive Suppressor T-Lymphocytes; #51 Suppressor T Lymphocytes, CD8 Positive; #52 Suppressor T-Lymphocyte, CD8-Positive; #53 T-Lymphocyte, CD8-Positive Suppressor; #54 T-Lymphocytes, CD8-Positive Suppressor; #55 Suppressor T-Cells, CD8-Positive; #56 CD8-Positive Suppressor T-Cell; #57 CD8-Positive Suppressor T-Cells; #58 Suppressor T Cells, CD8 Positive; #59 Suppressor T-Cell, CD8-Positive; #60 T-Cell, CD8-Positive Suppressor; #61 T-Cells, CD8-Positive Suppressor
#62 #31 or #32 or #33 or #34 or #35 or #36 or #37 or #38 or #39 or #40 or #41 or #42 or #43 or #44 or #45 or #46 or #47 or #48 or #49 or #50 or #51 or #52 or #53 or #54 or #55 or #56 or #57 or #58 or #59 or #60 or #61
#63 #30 or #62
#64 #8 and #63

Table 3
The complete searches in OVID.

Step no.	Search terms
1	Hepatitis B virus [Exp]
2	B virus, Hepatitis or Hepatitis B viruses or viruses, Hepatitis B or Hepatitis Virus, Homologous Serum or Dane Particle or Particle, Dane
3	1 or 2
4	T-Lymphocytes, Cytotoxic [Exp]
5	Cytotoxic T-Lymphocyte or T Lymphocytes, Cytotoxic or T-Lymphocyte, Cytotoxic or Cell-Mediated Lympholytic Cells or Cell Mediated Lympholytic Cells or Cell, Cell-Mediated Lympholytic or Cell-Mediated Lympholytic Cell or Cells, Cell-Mediated Lympholytic or Lympholytic Cell, Cell-Mediated or Lympholytic Cells, Cell-Mediated or Cytotoxic T-Lymphocytes or Cytotoxic T Lymphocytes or TC2 Cells or Cell, TC2 or Cells, TC2 or TC2 Cell or TC1 Cells or Cell, TC1 or Cells, TC1 or TC1 Cell
6	4 or 5
7	CD8-Positive T-Lymphocytes [Exp]
8	T-Lymphocyte, CD8-Positive or T-Lymphocytes, CD8-Positive or T8 Lymphocytes or Lymphocyte, T8 or Lymphocytes, T8 or T8 Lymphocyte or CD8-Positive Lymphocytes or CD8 Positive Lymphocytes or CD8-Positive Lymphocyte or Lymphocyte, CD8-Positive or Lymphocytes, CD8-Positive or T8 Cells or Cell, T8 or Cells, T8 or T8 Cell or Suppressor T-Lymphocytes, CD8-Positive or CD8-Positive Suppressor T-Lymphocyte or CD8-Positive Suppressor T-Lymphocytes or Suppressor T Lymphocytes, CD8 Positive or Suppressor T-Lymphocyte, CD8-Positive or T-Lymphocyte, CD8-Positive Suppressor or T-Lymphocytes, CD8-Positive Suppressor or Suppressor T-Cells, CD8-Positive or CD8-Positive Suppressor T-Cell or CD8-Positive Suppressor T-Cells or Suppressor T Cells, CD8 Positive or Suppressor T-Cell, CD8-Positive or T-Cell, CD8-Positive Suppressor or T-Cells, CD8-Positive Suppressor
9	7 or 8
10	6 or 9
11	3 and 10

fied by a search from January 1970 to July 2017. Studies were excluded if the full text could not be located or if the abstract was not informative.

2.2. Inclusion and exclusion of articles

We based inclusion and exclusion decisions on other studies [30–32]. Published studies in all languages were eligible, and the inclusion criteria were as follows: (1) we included studies comparing the courses of infection, seroconversion of HBeAg, levels of HBVDNA, or ALT activity levels in patients with or without HBV-specific effector $CD8^+$ T cells; (2) full texts (all languages) with an informative abstract in English; (3) a report of an HBV-specific effector $CD8^+$ T-cell level in peripheral blood, detected using major histocompatibility complex (MHC)-tetramer or MHC-pentamer; (4) appropriate study design: case-control, cross-sectional, or cohort studies or clinical trials; (5) the population of a study consisted of HBV mono-infected patients; (6) the decision for inclusion or exclusion was not influenced by results.

The exclusion criteria were as follows: (1) the patients of a study were coinfected with HBV and hepatitis C virus, hepatitis D virus, or human immunodeficiency virus; (2) HBV-specific effector $CD8^+$ T cells were detected after in vitro expansion; (3) conference proceedings, letters to the editor, and case reports; and (4) studies without control or comparison groups.

2.3. Data extraction and endpoint of interest

The data were extracted independently by two investigators; consensus was then reached or the article was referred to a third person. The information extracted from articles included the first author's name, publication year, country, characteristics of patients, the number of patients, HBV-specific effector $CD8^+$ T-cell level, course of infection, seroconversion of HBeAg, the level of HBVDNA, and ALT activity.

We compared the prognostic indicators between patients with and without HBV-specific effector $CD8^+$ T cells with no restrictions in terms of treatment history. The endpoint was a comparison of courses of the disease (acute HBV infection or CHB), the serum levels of HBVDNA in patients with CHB, seroconversion of HBeAg in patients with CHB, and activity of ALT in peripheral blood in specific-$CD8^+$-T-cell–positive patients compared to specific-$CD8^+$-T-cell–negative patients. We defined 10^7 copies/ml of HBVDNA as the cutoff level [33]; the cutoff of ALT was 70 U/L [34].

2.4. Statistical analysis

The odds ratios (ORs) and their 95% confidence intervals (CIs) were generated by the Mantel–Haenszel statistical method to determine the effects of specific $CD8^+$ T cells. Statistical heterogeneity of the results was quantified by the Q-test; a value of <0.10 was considered statistically significant. Additionally, the I^2 index was employed to assess the magnitude of heterogeneity [35], with values greater than 50% indicating moderate-to-high heterogeneity. The fixed- or random-effects model was used according to the results of the heterogeneity test. To assess the stability of overall prevalence, we performed a sensitivity analysis by reanalyzing the data after fixed/random effects model transformation. Analyses were performed in Review Manager 5.3.

3. Results

3.1. Literature review

Our electronic and manual search identified 1612 articles, of which 109 were deemed potentially relevant and were selected for full-text review. Then, 104 manuscripts were excluded for the following reasons: irrelevant, did not detect specific $CD8^+$ T cells by MHC-tetramer or -pentamer, did not provide the number of patients who were specific $CD8^+$ T-cell positive. Finally, five manuscripts were chosen for analysis [36–40]. The concordance between the reviewers regarding final inclusion and exclusion of studies was 100%. The flowchart in Fig. 1 highlights the articles election process.

3.2. Patients' characteristics

All included patients were HBV mono-infected, i.e., without HCV, HDV, or HIV coinfection. The baseline characteristics of studies included in the meta-analysis are given in Table 4, and the characteristics of patients enrolled in this analysis are shown in Table 5. There were two studies from China, two studies from Japan, and one study from the Netherlands. In this meta-analysis, 130 HBV-infected patients were enrolled. The mean age of patients varied from 24.4 to 36.11 years, and the gender distribution was as follows: the percentage of males ranged from 51.43% to 75%.

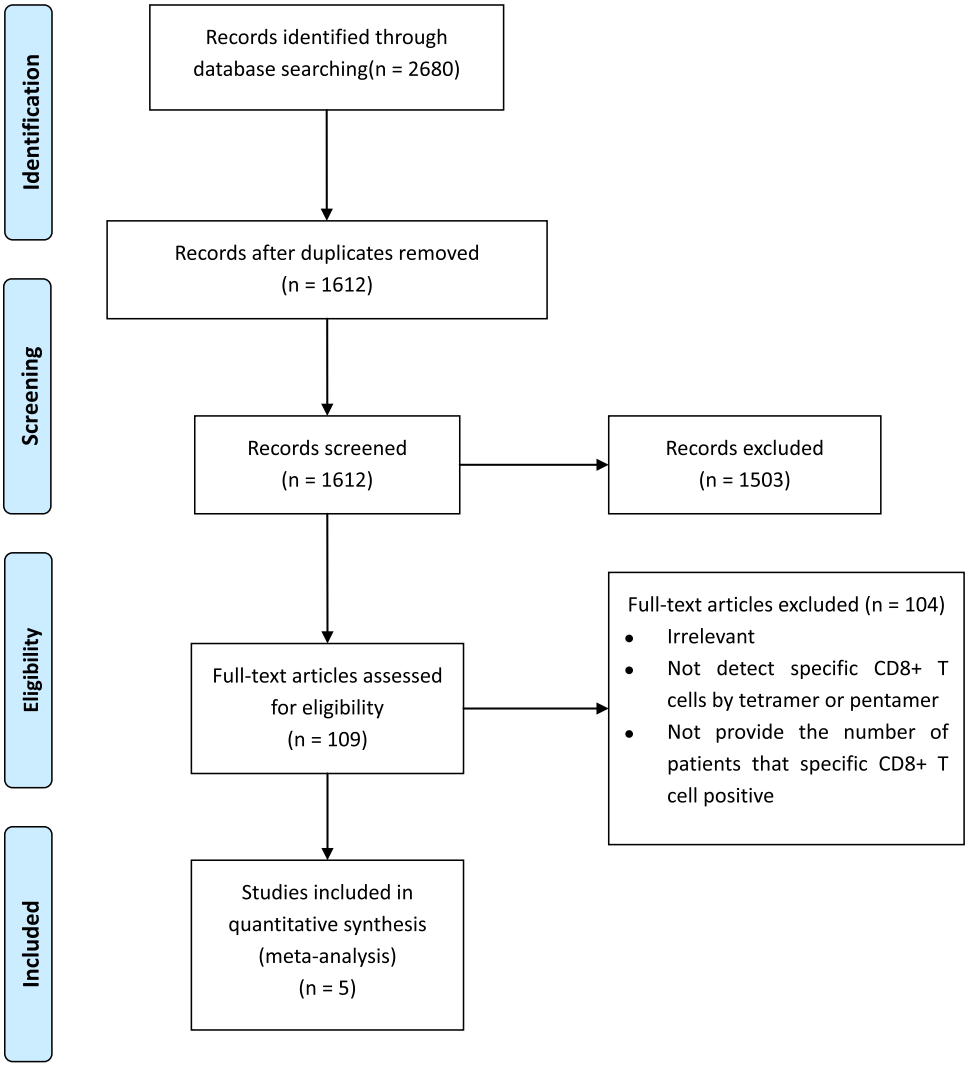

Fig. 1. The flowchart of the article inclusion process and meta-analysis.

Table 4
Baseline characteristics of studies included in the analysis.

Authors	Patients number	Mean age	Country	Publication year	Male (%)
Fan, Z. P.	20	24.4 ± 3.9/29.7 ± 2.5	China	2004	15 (75%)
Shimada, N.	35	36.11	Japan	2003	18 (51.43%)
Sobao, Y.	26	–	Japan	2002	–
Sprengers, D.	15	–	The Netherlands	2006	–
Yang, X.	34	35.71	China	2009	24 (70.59%)

Table 5
The characteristics of patients included in the analysis.

Authors	HBV prognosis		HBV DNA level		ALT level		HBeAg	
	Acute or self-limit	Chronic	$>10^7$ (copies/ml)	$<10^7$ (copies/ml)	>70 U/L	<70 U/L	HBeAg(+)	HBeAg(−)
Fan, Z. P.	7[1]	13	–	–	–	–	–	–
Shimada, N.	–	–	–	–	–	–	17	18
Sobao, Y.	13	13	8	5	–	–	6	7
Sprengers, D.	–	–	7	8	7	8	4	11
Yang, X.	16	18	7	11	12	22	8	10

[1] Number of patients in the article that were included for analysis.

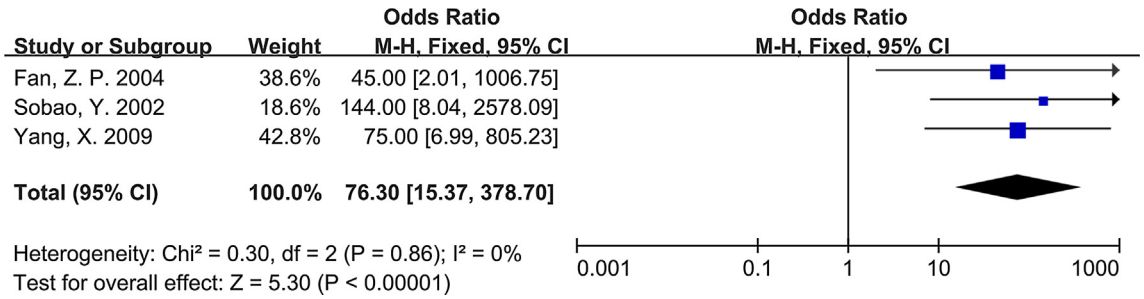

Fig. 2. Pooled odds ratios of effects of specific CD8⁺ T cells on the course of the disease (acute HBV infection versus CHB).

3.3. Effects of HBV-specific effector CD8⁺ T cells on the course of the disease

Specific CD8⁺ T cells are major contributors to viral clearance and are of life-changing importance for HBV-infected patients [41]. In this meta-analysis, patients with acute hepatitis B were found to have significantly increased levels of specific CD8⁺ T cells relative to patients with CHB. As depicted in Fig. 2, the pooled OR was 76.30 (95% CI: 15.37–378.70). The test of heterogeneity yielded nonsignificant results (P value of Q-squared = 0.86, I^2 = 0%); therefore, we selected the fixed-effects model. We reanalyzed the results using the random effects model; I^2 also turned out to be 0%, indicating no heterogeneity.

3.4. Effects of HBV-specific effector CD8⁺ T cells on the serum HBVDNA concentration

Specific CD8⁺ T cells can eliminate the virus and decrease HBVDNA levels through various mechanisms. According to our findings, among patients with CHB, patients with less than 10^7 copies/ml HBVDNA had higher levels of specific CD8⁺ T cells than did patients with more than 10^7 copies/ml HBVDNA, but the difference had no statistics significance. (OR: 3.89, 95% CI: 0.71–21.33). The test of heterogeneity yielded nonsignificant results (P value of Q-squared = 0.83, I^2 = 0%); therefore, we chose the fixed model. The results are presented in Fig. 3. We reanalyzed the results via the random-effects model; I^2 was also 0%, indicating no heterogeneity.

3.5. Effects of HBV-specific effector CD8⁺ T cells on serum ALT activity

Based on our findings, there was no significant association between the levels of specific CD8⁺ T cells and serum ALT activity. As depicted in Fig. 4, the pooled OR was 0.86 (95% CI: 0.01–74.15). The test of heterogeneity gave a significant result (P value of Q-squared = 0.01, I^2 = 84%); therefore, we chose the random model. The results are shown in Fig. 4. We reanalyzed the results by means of the fixed-effects model; I^2 was found to be 84%, indicating that the heterogeneity was significant.

3.6. Effects of HBV-specific effector CD8⁺ T cells on seroconversion of HBeAg

Specific CD8⁺ T cells are a major participant in HBV infection, whereas HBeAg seroconversion is an important milestone in the management of HBeAg-positive patients with CHB [42]. It has been reported that HBeAg persistence is associated with the properties of HBV-specific effector CD8⁺ T cells [43]. According to the present meta-analysis, among patients with CHB, patients with negative HBeAg or positive anti-HBeAg antibody (anti-HBe) results had significantly higher levels of specific CD8⁺ T cells than did patients positive for HBeAg (OR: 5.82, 95% CI: 1.41–24.13). The test of heterogeneity yielded nonsignificant results (P value of Q-squared = 0.95, I^2 = 0%); therefore, we chose the fixed model. The results are presented in Fig. 5. We reanalyzed the results via the random-effects model; I^2 also turned out to be 0%, indicating no heterogeneity.

4. Discussion

The mechanisms of progression of HBV infection remain unclear. Functionally competent antiviral T-cell responses are major contributors to the elimination of HBV during acute infection. In CHB, the presence of dysfunctional HBV-specific effector CD8⁺ T cells results in persistent infection by HBV [16,44,45]; therefore, HBV-specific effector CD8⁺ T cells may affect the prognosis of HBV infection. Nonetheless, one study [46] has revealed that the frequency of HBV-specific effector CD8⁺ T cells is similar among asymptomatic HBV-infected individuals, individuals with active chronic flared hepatitis B, and individuals with HBV-infected HCC. In addition, the serum levels of HBVDNA do not correlate with the frequency of CD8⁺ T cells specific to HBV [46]. Consequently, the correlation between HBV-specific effector CD8⁺ T cells and the prognosis of HBV infection remains a controversial topic;

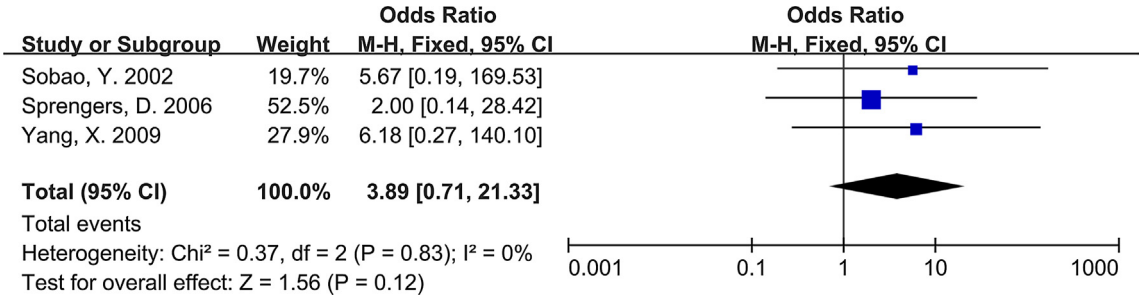

Fig. 3. . Pooled odds ratios of effects of specific CD8⁺ T cells on viral replication (patients with <10^7 copies/ml HBVDNA versus patients >10^7 copies/ml HBVDNA) among patients with CHB.

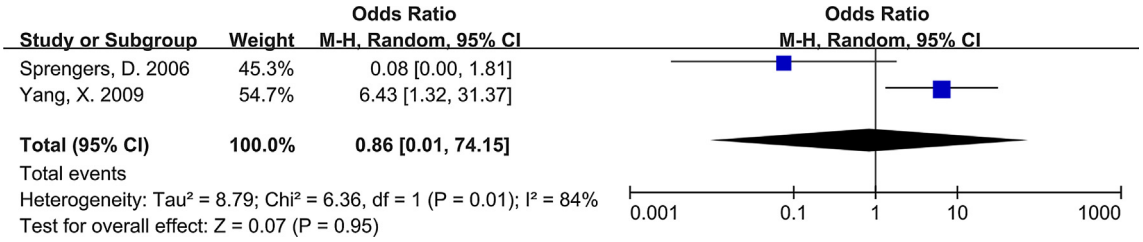

Fig. 4. Pooled odds ratios of effects of specific CD8$^+$ T cells on serum ALT activity (patients with ALT < 70 U/L versus patients with ALT > 70 U/L).

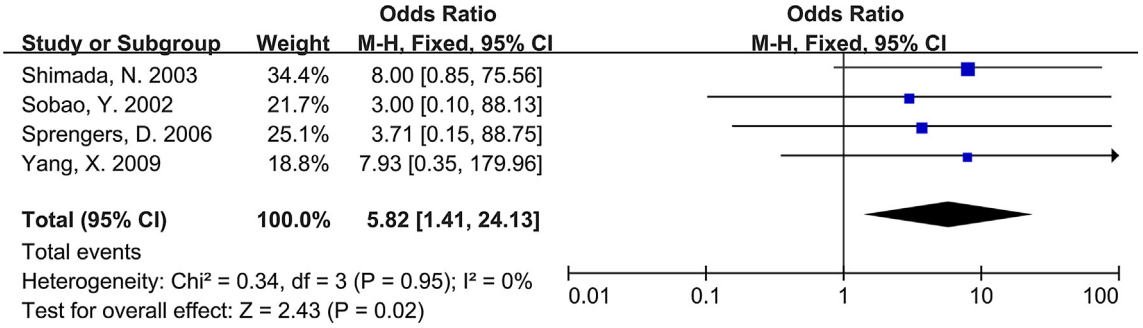

Fig. 5. Pooled odds ratios of effects of specific CD8$^+$ T cells on HBeAg seroconversion (patients with negative HBeAg or positive anti-HBe results versus patients positive for HBeAg) among patients with CHB.

thus, we investigated the pooled effects of HBV-specific effector CD8$^+$ T cells on HBV infection.

CD8$^+$ T cells can control replication of HBV and eliminate the virus via cytolytic and noncytolytic mechanisms [47,48]. HBV-specific CD8$^+$ T cells can kill HBV infected hepatocytes through direct lysis, which is mediated by perforin and granzyme. Then, the contents of HBV are released to serum. Finally, the antibody against HBV and innate immune response will eliminate the contents of HBV. HBV-specific CD8$^+$ T cells also can eliminate virus through noncytolytic method, and the mechanism is unknown. Therefore, specific CD8$^+$ T cells may play a role in resolving HBV infection and preventing persistent HBV infection. In this meta-analysis, HBV-specific effector CD8$^+$ T cells were found to be more abundant in patients with acute hepatitis B than in patients with CHB. However, this difference can be induced by many factors, such as high viral load during acute infection and immune state of host. Therefore, further studies are needed to explore causation of this difference.

The relaxed circular DNA is reverse-transcribed from pregenomic RNA, which depends on virus replication [49]. In addition, cccDNA is formed from relaxed circular DNA during the lifecycle of the virus [50]. Therefore, when specific CD8$^+$ T cells control HBV replication [47], the viral-DNA level decreases. In light of the data from our meta-analysis, among patients with CHB, patients with low HBVDNA levels had a higher level of specific CD8$^+$ T cells than did patients with high HBVDNA levels, but the difference had no statistics significance. This situation might result from too few studies we included. Thus, further studies are needed.

Moreover, specific CD8$^+$ T cells perform a major function in an adaptive immune response, which is critical for elimination of viral antigens and for resolving HBV infection [51]. The seroconversion of HBeAg is an indicator of disease progression. Theoretically, the levels of specific CD8$^+$ T cells should negatively correlate with the level of HBeAg. In this meta-analysis, among patients with CHB, the level of specific CD8$^+$ T cells was higher in HBeAg$^-$ or anti-HBe$^+$ patients than in HBeAg$^+$ patients. This result implied that specific CD8$^+$ T cells might contribute to eliminate viral antigens.

Recognition of infected hepatocytes by HBV-specific effector CD8$^+$ T cells has been considered the primary mechanism underlying both virus control and liver damage [52,53]; thus, large numbers of HBV-specific effector CD8$^+$ T cells should increase serum ALT activity through damage to infected hepatocytes. Nevertheless, the authors of another study [34] believe that in the presence of effective HBV-specific effector CD8$^+$ T cells, inhibition of virus replication can be independent of liver damage. In addition, liver pathology is caused by the recruitment of non–virus-specific T cells [34]. Therefore, the relation between the numbers of HBV-specific effector CD8$^+$ T cells and serum ALT activity is indeterminate. The results of this meta-analysis also indicate that there is no significant correlation between the levels of specific CD8$^+$ T cells and serum ALT activity. Authors of one article [40] included in this meta-analysis have concluded that specific CD8$^+$ T cells may reduce ALT levels, whereas another study [39] has shown that the correlation between the levels of specific CD8$^+$ T cells and serum ALT activity is not significant. Further large-scale studies are necessary to resolve this discrepancy.

Another meta-analysis indicates that Tregs are a determinant of HBV infection prognosis and reveals that Tregs influence the disease activity in HBV-infected patients in various ways [30]. The correlation between the genetic polymorphisms of the cytotoxic T-lymphocyte-associated protein 4 (CTLA4) and HBV clearance has also been assessed by meta-analysis [54]. On the other hand, the relation between the levels of HBV-specific effector CD8$^+$ T cells and the progression or prognosis of HBV infection has never been analyzed by meta-analysis. It is well known that Tregs are CD4$^+$, whereas virus-specific effector T cells are CD8$^+$, suggesting that virus-specific CD8$^+$ T cells can not be Tregs. Our study leads to the conclusion that HBV-specific effector CD8$^+$ T cells are an important protective factor in the progression and prognosis of HBV infection and confirms the importance of studies aimed at inducing specific T-cell immune responses to cure CHB.

At the beginning of this study, we wanted to evaluate the correlation between specific CD8$^+$ T cells and all the indicators related to HBV progression; however, some indicators were removed from

the analysis because relevant studies were lacking, e.g., those related to HCC and a response to IFN-α treatment. Eventually, we chose four indicators: acute or chronic infection, seroconversion of HBeAg, levels of HBVDNA, and serum ALT activity. We designated 10^7 copies/ml as the cutoff between high and low DNA levels according to other research [33]. In addition, the cutoff level for serum ALT activity was set to 70 U/L because histological diagnosis revealed that patients with ALT > 70 U/L are thought to have chronic active hepatitis with massive portal infiltration of mononuclear cells [34].

Just as any systematic reviews and meta-analyses, this study has several limitations. First, although we tried our best to include all appropriate studies, articles that matched the inclusion criteria were still too few; this situation ruled out adequate subgroup analysis. Additionally, the number of patients included in this meta-analysis is small, which clearly precludes more definitive conclusions. Second, individual data were not available in some studies, thereby limiting the performance of our own adjustments. Third, this study may be affected by publication bias because negative trials are less likely to be published. We utilized various strategies to search for published and unpublished trials to limit the effects of publication bias. An additional limitation of this meta-analysis is that we selected only studies where researchers used MHC-tetramer [55,56] or MHC-pentamer [57] to detect HBV-specific effector $CD8^+$ T cells; this approach might have caused a selection bias.

5. Conclusions

The present study is the first meta-analysis indicating that HBV-specific effector $CD8^+$ T cells are an important protective factor in the progression and prognosis of HBV infection and confirms the importance of current studies aimed at inducing specific T-cell immune responses. Specific $CD8^+$ T cells influence disease prognosis by preventing persistent infection, by reducing the concentration of HBVDNA, and by promoting seroconversion from HBeAg to anti-HBe. Nevertheless, the correlation between the levels of specific $CD8^+$ T cells and serum ALT activity should be investigated further. Therefore, HBV-specific effector $CD8^+$ T cells are an important endpoint on which immunotherapy should be focused.

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Declarations of interest

None.

Disclosure

Author Contributions: J.W. and J.Z. conceived of and designed the study; Z.O.,Y.X., and Z.X. searched for the data; X.L. and S.J. analyzed the data; Y.L., J.Z., and J.W. wrote the manuscript.

References

[1] Hong X, Kim ES, Guo H. Epigenetic regulation of hepatitis B virus covalently closed circular DNA: implications for epigenetic therapy against chronic hepatitis B. Hepatology 2017;66:2066–77.
[2] Allain JP, Opare-Sem O. Screening and diagnosis of HBV in low-income and middle-income countries. Nat Rev Gastroenterol Hepatol 2016;13:643–53.
[3] Petersen J, Thompson AJ, Levrero M. Aiming for cure in HBV and HDV infection. J Hepatol 2016;65:835–48.
[4] Revill P, Testoni B, Locarnini S, Zoulim F. Global strategies are required to cure and eliminate HBV infection. Nat Rev Gastroenterol Hepatol 2016;13:239–48.
[5] Durantel D, Zoulim F. New antiviral targets for innovative treatment concepts for hepatitis B virus and hepatitis delta virus. J Hepatol 2016;64:S117–31.
[6] Zeisel MB, Lucifora J, Mason WS, Sureau C, Beck J, Levrero M, et al. Towards an HBV cure: state-of-the-art and unresolved questions–report of the ANRS workshop on HBV cure. Gut 2015;64:1314–26.
[7] Feld JJ, Colledge D, Sozzi V, Edwards R, Littlejohn M, Locarnini SA. The phenylpropenamide derivative AT-130 blocks HBV replication at the level of viral RNA packaging. Antiviral Res 2007;76:168–77.
[8] Stray SJ, Bourne CR, Punna S, Lewis WG, Finn MG, Zlotnick A. A heteroaryldihydropyrimidine activates and can misdirect hepatitis B virus capsid assembly. Proc Natl Acad Sci USA 2005;102:8138–43.
[9] Cai D, Mills C, Yu W, Yan R, Aldrich CE, Saputelli JR, et al. Identification of disubstituted sulfonamide compounds as specific inhibitors of hepatitis B virus covalently closed circular DNA formation. Antimicrob Age Chemother 2012;56:4277–88.
[10] Schiffer JT, Swan DA, Stone D, Jerome KR. Predictors of hepatitis B cure using gene therapy to deliver DNA cleavage enzymes: a mathematical modeling approach. PLoS Comput Biol 2013;9:e1003131.
[11] Chen J, Zhang W, Lin J, Wang F, Wu M, Chen C, et al. An efficient antiviral strategy for targeting hepatitis B virus genome using transcription activator-like effector nucleases. Molecul Thera J Am Soc Gene Thera 2014;22:303–11.
[12] Billioud G, Kruse RL, Carrillo M, Whitten-Bauer C, Gao D, Kim A, et al. In vivo reduction of hepatitis B virus antigenemia and viremia by antisense oligonucleotides. J Hepatol 2016;64:781–9.
[13] Nkongolo S, Ni Y, Lempp FA, Kaufman C, Lindner T, Esser-Nobis K, et al. Cyclosporin A inhibits hepatitis B and hepatitis D virus entry by cyclophilin-independent interference with the NTCP receptor. J Hepatol 2014;60:723–31.
[14] Lucifora J, Xia Y, Reisinger F, Zhang K, Stadler D, Cheng X, et al. Specific and nonhepatotoxic degradation of nuclear hepatitis B virus cccDNA. Science 2014;343:1221–8.
[15] Liu J, Zhang E, Ma Z, Wu W, Kosinska A, Zhang X, et al. Enhancing virus-specific immunity in vivo by combining therapeutic vaccination and PD-L1 blockade in chronic hepadnaviral infection. PLoS Pathog 2014;10:e1003856.
[16] Bengsch B, Martin B, Thimme R. Restoration of HBV-specific CD8+ T cell function by PD-1 blockade in inactive carrier patients is linked to T cell differentiation. J Hepatol 2014;61:1212–9.
[17] King TH, Kemmler CB, Guo Z, Mann D, Lu Y, Coeshott C, et al. A whole recombinant yeast-based therapeutic vaccine elicits HBV X, S and Core specific T cells in mice and activates human T cells recognizing epitopes linked to viral clearance. PloS one 2014;9:e101904.
[18] Lampertico P, Maini M, Papatheodoridis G. Optimal management of hepatitis B virus infection - EASL Special Conference. J Hepatol 2015;63:1238–53.
[19] Bertoletti A, Ferrari C. Adaptive immunity in HBV infection. J Hepatol 2016;64:S71–83.
[20] Fioravanti J, Di Lucia P, Magini D, Moalli F, Boni C, Benechet AP, et al. Effector CD8+ T cell-derived interleukin-10 enhances acute liver immunopathology. J Hepatol 2017;67:543–8.
[21] Benechet AP, Iannacone M. Determinants of hepatic effector CD8+ T cell dynamics. J Hepatol 2017;66:228–33.
[22] Park JJ, Wong DK, Wahed AS, Lee WM, Feld JJ, Terrault N, et al. Hepatitis B virus-specific and global T-Cell dysfunction in chronic hepatitis B. Gastroenterology 2016;150(684–95):e5.
[23] Kosinska AD, Pishraft-Sabet L, Wu W, Fang Z, Lenart M, Chen J, et al. Low hepatitis B virus-specific T-cell response in males correlates with high regulatory T-cell numbers in murine models. Hepatology 2017;66:69–83.
[24] Wisskirchen K, Metzger K, Schreiber S, Asen T, Weigand L, Dargel C, et al. Isolation and functional characterization of hepatitis B virus-specific T-cell receptors as new tools for experimental and clinical use. PloS one 2017;12:e0182936.
[25] Trandem K, Zhao J, Fleming E, Perlman S. Highly activated cytotoxic CD8 T cells express protective IL-10 at the peak of coronavirus-induced encephalitis. J Immunol 2011;186:3642–52.
[26] Puntambekar SS, Bergmann CC, Savarin C, Karp CL, Phares TW, Parra GI, et al. Shifting hierarchies of interleukin-10-producing T cell populations in the central nervous system during acute and persistent viral encephalomyelitis. J Virol 2011;85:6702–13.
[27] Wherry EJ, Sun J, Cardani A, Sharma AK, Laubach VE, Jack RS, et al. Autocrine regulation of pulmonary inflammation by effector T-cell derived IL-10 during infection with respiratory syncytial virus. PLoS Pathog 2011;7:e1002173.
[28] Sun J, Madan R, Karp CL, Braciale TJ. Effector T cells control lung inflammation during acute influenza virus infection by producing IL-10. Nat Med 2009;15:277–84.
[29] Barnes E. Therapeutic vaccines in HBV: lessons from HCV. Med Microbiol Immunol 2015;204:79–86.
[30] Aalaei-Andabili SH, Alavian SM. Regulatory T cells are the most important determinant factor of hepatitis B infection prognosis: a systematic review and meta-analysis. Vaccine 2012;30:5595–602.
[31] Fabrizi F, Dixit V, Messa P, Martin P. Hepatitis B virus vaccine in chronic kidney disease: improved immunogenicity by adjuvants? A meta-analysis of randomized trials. Vaccine 2012;30:2295–300.
[32] El Sherbini A, Omar A. Treatment of children with HBeAg-positive chronic hepatitis B: a systematic review and meta-analysis. Digest Liver Dis Off J Italian Soc Gastroenterol Italian Assoc Study Liver 2014;46:1103–10.
[33] Webster GJ, Reignat S, Brown D, Ogg GS, Jones L, Seneviratne SL, et al. Longitudinal analysis of CD8+ T cells specific for structural and nonstructural

hepatitis B virus proteins in patients with chronic hepatitis B: implications for immunotherapy. J Virol 2004;78:5707–19.
[34] Maini MK, Boni C, Lee CK, Larrubia JR, Reignat S, Ogg GS, et al. The role of virus-specific CD8(+) cells in liver damage and viral control during persistent hepatitis B virus infection. J Exp Med 2000;191:1269–80.
[35] Petitti DB. Approaches to heterogeneity in meta-analysis. Stat Med 2001;20:3625–33.
[36] Fan ZP, Wang FS, Xu DP, Chu FL, Shi M, Zhou Y, et al. Detection of HBcAg-specific cytotoxic lymphocytes and their association with clinical status in patients with hepatitis B. Zhonghua yi xue za zhi 2004;84:2073–6.
[37] Shimada N, Yamamoto K, Kuroda MJ, Terada R, Hakoda T, Shimomura H, et al. HBcAg-specific CD8 T cells play an important role in virus suppression, and acute flare-up is associated with the expansion of activated memory T cells. J Clin Immunol 2003;23:223–32.
[38] Sobao Y, Tomiyama H, Sugi K, Tokunaga M, Ueno T, Saito S, et al. The role of hepatitis B virus-specific memory CD8 T cells in the control of viral replication. J Hepatol 2002;36:105–15.
[39] Sprengers D, Van Der Molen RG, Kusters JG, Hansen B, Niesters HGM, Schalm SW, et al. Different composition of intrahepatic lymphocytes in the immune-tolerance and immune-clearance phase of chronic hepatitis B. J Med Virol 2006;78:561–8.
[40] Yang X, Hao Y, Liu Z, Chen L, Ding H, Zhao X, et al. Frequencies and characterization of HBV-specific cytotoxic T lymphocytes in self-limited and chronic hepatitis B viral infection in China. J Huazhong Univ Sci Technol - Med Sci 2009;29:567–74.
[41] Nitschke K, Luxenburger H, Kiraithe MM, Thimme R, Neumann-Haefelin C. CD8+ T-cell responses in hepatitis B and C: the (HLA-) A, B, and C of hepatitis B and C. Dig Dis 2016;34:396–409.
[42] Liaw YF. HBeAg seroconversion as an important end point in the treatment of chronic hepatitis B. Hep Intl 2009;3:425–33.
[43] Peng G, Luo B, Li J, Zhao D, Wu W, Chen F, et al. Hepatitis B e-antigen persistency is associated with the properties of HBV-specific CD8 T cells in CHB patients. J Clin Immunol 2011;31:195–204.
[44] Maini MK, Schurich A. The molecular basis of the failed immune response in chronic HBV: therapeutic implications. J Hepatol 2010;52:616–9.
[45] Bertoletti A, Ferrari C. Innate and adaptive immune responses in chronic hepatitis B virus infections: towards restoration of immune control of viral infection. Gut 2012;61:1754–64.
[46] Dinney CM, Zhao LD, Conrad CD, Duker JM, Karas RO, Hu Z, et al. Regulation of HBV-specific CD8(+) T cell-mediated inflammation is diversified in different clinical presentations of HBV infection. J Microbiol 2015;53:718–24.
[47] Phillips S, Chokshi S, Riva A, Evans A, Williams R, Naoumov NV. CD8(+) T cell control of hepatitis B virus replication: direct comparison between cytolytic and noncytolytic functions. J Immunol 2010;184:287–95.
[48] Guidotti LG, Ishikawa T, Hobbs MV, Matzke B, Schreiber R, Chisari FV. Intracellular inactivation of the hepatitis B virus by cytotoxic T lymphocytes. Immunity 1996;4:25–36.
[49] Grimm D, Thimme R, Blum HE. HBV life cycle and novel drug targets. Hep Intl 2011;5:644–53.
[50] Nassal M. HBV cccDNA: viral persistence reservoir and key obstacle for a cure of chronic hepatitis B. Gut 2015;64:1972–84.
[51] Fisicaro P, Valdatta C, Massari M, Loggi E, Ravanetti L, Urbani S, et al. Combined blockade of programmed death-1 and activation of CD137 increase responses of human liver T cells against HBV, but not HCV. Gastroenterology 2012;143 (1576–85):e4.
[52] Thimme R, Wieland S, Steiger C, Ghrayeb J, Reimann KA, Purcell RH, et al. CD8+ T cells mediate viral clearance and disease pathogenesis during acute hepatitis B virus infection. J Virol 2003;77:68–76.
[53] Chisari FV, Ferrari C. Hepatitis B virus immunopathogenesis. Annu Rev Immunol 1995;13:29–60.
[54] Xu H, Zhao M, He J, Chen Z. Association between cytotoxic T-lymphocyte associated protein 4 gene +49 A/G polymorphism and chronic infection with hepatitis B virus: a meta-analysis. J Int Med Res 2013;41:559–67.
[55] Li S, Mwakalundwa G, Skinner PJ. In situ MHC-tetramer staining and quantitative analysis to determine the location, abundance, and phenotype of antigen-specific CD8 T cells in tissues. J Visual Experim JoVE 2017;127: e56130.
[56] Fehlings M, Chakarov S, Simoni Y, Sivasankar B, Ginhoux F, Newell EW. Multiplex peptide-MHC tetramer staining using mass cytometry for deep analysis of the influenza-specific T-cell response in mice. J Immunol Meth 2017;453:30–6.
[57] Tan WG, Zubkova I, Kachko A, Wells F, Adler H, Sutter G, et al. Qualitative differences in cellular immunogenicity elicited by hepatitis C virus T-Cell vaccines employing prime-boost regimens. PloS one 2017;12:e0181578.