

Supplementary

Table S1. Search table for the review question: “which role the CTLA-4 receptor plays in the exhaustion state in T-cells seen in patients chronically infected with HBV”.

OR	AND	
	CTLA-4	Hepatitis B
	CTLA-4 antigen (MeSH)	Hepatitis B (MeSH)
	CTLA-4 (Text word)	Hepatitis B, chronic (MeSH)
	Cytotoxic-lymphocyte-associated antigen 4 (Text word)	Hepatitis B virus infection (Text word)
	CD152 (Text word)	Chronic hepatitis B (Text word)
		Chronic HBV (Text word)
		Persistent HBV infection (Text word)
		CHB patients (Text word)
		Hepatitis B (Text word)

Search Pubmed:

((("CTLA-4 Antigen"[Mesh Terms] OR (((Cytotoxic T lymphocyte-associated antigen-4[Text Word] OR (CTLA-4[Text Word])) OR (CD152[Text Word])))) AND (((("Hepatitis B"[Mesh Terms]) OR "Hepatitis B, Chronic"[Mesh Terms]) OR ((((((Hepatitis B Virus Infection[Text Word]) OR (Chronic Hepatitis B[Text Word])) OR (Chronic HBV[Text Word])) OR (persistent HBV infection[Text Word])) OR (CHB patients[Text Word])) OR (Hepatitis B[Text Word]))))

Search Embase:

Link to search on OVID:

<https://ep.fjernadgang.kb.dk/login?url=http://ovidsp.ovid.com/ovidweb.cgi?T=JS&NEWS=N&PAGE=main&SHAREDSEARCHID=4NVOLjOOznE3eZvkzlbA7WkkUUsY1kq0brTx0wmv7b5RDoK0AVsP54KIZvV6nKFTD>

1. exp cytotoxic T lymphocyte antigen 4/
2. CTLA-4.mp. [mp=title, abstract, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword, floating subheading word, candidate term word]
3. Cytotoxic-lymphocyte-associated antigen 4.mp. [mp=title, abstract, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword, floating subheading word, candidate term word]
4. CD152.mp. [mp=title, abstract, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword, floating subheading word, candidate term word]
5. exp Hepatitis B virus/ or exp hepatitis B/
6. Hepatitis B, chronic.mp. or exp chronic hepatitis B/
7. Hepatitis B virus infection.mp. [mp=title, abstract, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword, floating subheading word, candidate term word]
8. Chronic HBV.mp. [mp=title, abstract, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword, floating subheading word, candidate term word]

9. Persistent HBV infection.mp. [mp=title, abstract, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword, floating subheading word, candidate term word]

10. CHB patient.mp. [mp=title, abstract, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword, floating subheading word, candidate term word]

11. CHB.mp. [mp=title, abstract, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword, floating subheading word, candidate term word]

12. 1 or 2 or 3 or 4

13. 5 or 6 or 7 or 8 or 9 or 10 or 11

14. 12 and 1

Table S2_Overview of included studies. CD8+ T-cells

Source	Aim	Study participants	Inclusion criteria	Exclusion criteria	Main T-cell type	Global - or HBV specific T cells	Primary findings
Bensch, B, [21] (2014)	To investigate upregulation of inhibitory receptors on HBV-specific CD8+ T-cells and effect of PD-1 blockade	Total CHB: n= 98 HBV-specific CD8+ T-cell responses: n = 22 CHB liver biopsy: n = 6	HBsAg-positive HLA-A*02 positive	Antiviral therapy HCV or HDV infection HCC Patients without full virological data	CD8+	HBV specific	PD-1 was the primary inhibitory receptor on HBV-specific CD8+ from peripheral blood and liver biopsies. CTLA-4 expression was significantly weaker (21.4%) Response to inhibitory receptor blockade was heterogeneous. The strongest increase in proliferation was observed in PD-L1 blockade (184%), while CTLA-4 blockade was less effective (53%).
Jiang, D. [22] (2022)	To investigate the phenotypic heterogeneity of exhausted CD8+ T cells in HBV	CHB: n = 31 HC: n = 23	HBsAg positive for > 6 months Normal liver function	HAV, HCV, HDV infection Normal liver function Other severe or active diseases	CD8+	Global	CD8+ T cells exhibited higher levels of inhibitory receptors (CTLA-4, LAG3, TIM3 and PD1) in CHB patients than in HCs, but only PD1 and TIM3 were significantly increased mRNA expression of CTLA4 was increased in the PBMCs of CHB patients CTLA4 expression was slightly elevated in CXCR5+CD8+ T cells compared to CXCR5-CD8+ T cells CD8+ T cells produced lower levels of cytokines (IFN- γ , TNF- α , and Granzyme B).

Park, J. J. [23] (2016)	To examine whether T-cell effector and regulatory responses can define clinical stages of CHB	CHB: n = 200 HC: n = 20	HBsAg-positive ≥ 18 years	Antiviral therapy HIV infection HCC Hepatic decompensation Liver transplant Active autoimmune disease Medications, or comorbid illnesses that can impact immune response.	CD8+ and CD4+	HBV specific (+ Flu specific)	Antiviral T-cell responses do not provide distinct immune signatures for CHB phenotypes. No difference in level of CTLA-4 and PD-1 expression in CD8+T-cells in CHB patients compared to HC.
Peng, G., Luo, B. et al [24] (2011)	To characterize the association between persistent HBeAg and the properties of HBV- specific CD8 T cells, as well as the levels of liver injury in CHB patients with different HBeAg statuses.	CHB: n = 103 HC: n = 30	CHB diagnosis HLA-A2 positive	Antiviral - or steroid therapy past 6 months HCV, HDV, or HIV infection Other markers of autoimmune hepatitis and drug-induced hepatitis.	CD8+	HBV specific	Differences in HBV-pentamer+ T cell frequency were not significant, but increased CTLA-4 (and PD-1 expression) on HBV-specific CD8 T cells was seen in the HBeAg+ group. HBV-peptide stimulation with anti-CTLA-4 and anti-PD-L1 significantly increased the proliferation in PBMCs, but enhanced IFN- γ production only in the HBeAg+ patients.
Schurich, A [26] (2011)	Examine the propensity of CD8+ T-cells from patients with CHB to up-	CHB: n = 86 RHB: n = 3 HC = 23	CHB diagnosis Treatment-naïve at recruitment CMV seropositive	HCV or HIV infection	CD8+	Both	CD8+ T-Cells in CHB had an enhanced propensity to up-regulate CTLA-4. Correlation between HBV DNA and CTLA-4 expression on global CD8+ T cells.

	regulate CTLA-4.						The level of CTLA-4 expression on HBV-specific CD8+ T cells was increased compared to the total CD8+ T cell population Correlation between increased CTLA-4 and Bim on HBV-specific CD8+ T-cells.
Tang, ZS [28] (2016)	To investigate the expression characteristics of CD28 family on T-cells in patients with CHB and to investigate the effects of PD-1 blockade	CHB: n = 52 HC: n = 26	HBsAg-positive	Antiviral - or immune-suppressant therapy past 1 year Other liver diseases	CD8+ and CD4+	Global	No increased expression of CTLA-4 on CD8+ T-cells compared to HC. No correlation between viral load and increased expression of CTLA-4 on CD8+ T-cells.
Wang, X [9] (2019)	To explore the genetic and phenotypic difference in CD8+ T-cell exhaustion between CHB patients and HCC.	CHB/HCC: 40 (liver tissue) HC: n = 40 (PBMCs)	NA	NA	CD8+	Global	Increased expression of CTLA-4, PD-1, Tim-3, LAG-3 in CHB and HCC liver tissue Reduced CD8+ T-cell function and reduced production of IL-2, IFN-γ and TNF-α in CHB and HCC samples. CD8+ T cell exhaustion existed in both CHB and HCC, but the phenotypes, functional states and underlying mechanisms are different between the two.

Yu, Y [11] (2009)	To test the efficacy of RNAi against CTLA4 in human PBMCs of CHB patients and to determine whether there was any difference in the Th-responses after siRNA transfection.	CHB: n = 120	HBsAg + anti-HBcAb- positive for > 6 months	Antiviral - or immune-suppressive therapy HCV, HDV or HIV infection History of alcoholism Patients who did not participate in all phases of the study protocol	Lympho-cytes (PBMCs)	Global	A significant positive correlation between CTLA4 mRNA and HBV DNA mRNA levels. siRNAs could downregulate the mRNA expression of CTLA4. The expressions of IFN-γ and IL-2 were upregulated in lymphocytes transfected with siRNA-1.
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CHB = chronic hepatitis B virus infection; CHB pre = pre-treatment; CHB post = post treatment with antiviral therapy; HC = healthy controls; RHB = resolved HBV infection; HCC = hepatocellular carcinoma; HIV = human immunodeficiency virus; HCV = hepatitis C virus; HDV hepatitis D virus; CMV = cytomegalovirus; NA = nucleos(t)ide analogs; PBMC = peripheral blood mononuclear cells; seq-IFN = sequential interferon therapy; VL = viral load

Table S3. Overview of included studies. CD4+ T-cells

Source	Aim	Study participants (n)	Inclusion criteria	Exclusion criteria	Main T-cell type	Global - or HBV specific T cells	Primary findings*
Park, J. J. [23] (2016)	To examine whether T-cell effector and regulatory responses can define clinical stages of CHB	CHB: n = 200 HC: n = 20	HBsAg-positive ≥ 18 years	Antiviral therapy HIV infection HCC Hepatic decompensation Liver transplant Autoimmune disease Medications, or comorbid illnesses that can impact immune response	CD4+ and CD8+	HBV specific (+ Flu specific)	CTLA-4 expression levels in CD4+ T-cells were greater in CHB patients than in HC
Raziourrouh, B. [25] (2014)	To characterize the memory and inhibitory phenotype of virus-specific CD4+ T- cells during CHB and the functional impact of negative regulatory molecules	CHB: n = 66 AHB: n = 41 RHB: n = 5 HC: n = 5	HBsAg + anti-HBcAb-positive > 6 months	HCV, HDV or HIV infection	CD4+	HBV specific	CD4+ T-cells most frequently expressed PD-1 (77.9%) in contrast to CTLA-4 (19.6%) CTLA-4 blockade did not increase proliferation of HBV-specific CD4+ T-cells.
Tang, ZS [28] (2016)	To investigate the expression	CHB: n = 52 HC: n = 26	HBsAg-positive	Antiviral or immunosuppressan	CD4+ and CD8+	Global	Levels of CTLA-4 on CD4+ T-cells were increased in CHB patients

	characteristics of CD28 family on T-cells in patients with CHB and to investigate the effects of PD-1 blockade			t therapy the past 1 year. Other chronic liver diseases			No correlations between virological parameters and the abnormal expression of the CD28 family receptors on CD4+T-cells Following anti-PD-L1 exposure, the expression levels of CD28, ICOS, PD-1 and CTLA-4 were increased in the CD4+ T-cells
Wang, L. [29] (2014)	To investigate the correlation between the expression levels of costimulatory molecules and the different states of CHB infection, including expression levels before and following antiviral treatment.	CHB pre: n = 30 CHB post: n = 32 HC: n = 30	HBsAg-positive	HCV, HDV or HIV infection Other causes of chronic liver damage	CD4+	Global	Level of CD4+ significantly decreased in CHB patients compared to HC The expression levels of CTLA-4 on CD4+ T-cells were significantly lower in the two CHB groups compared to HC No correlation between viral load and CTLA-4 expression levels.
Wen, C. [26] (2023)	To investigate the role of CD4+CXCR5-FOXP+ T cells with CTLA4 expression in patients with CHB	CHB (treatment naïve): n = 106 HC: n = 25	CHB	HAV, HCV, HDV, HEV or HIV infection Autoimmune diseases Other severe or active disease	CD4+	Global	No significant difference in CTLA4 expression in CXCR5-FOXP3+ cells compared to CXCR5+FOXP3+ cells . Comparable CTLA4 expression levels were observed in circulating and splenic CD4+CXCR5-FOXP3+ T cells.

		HBV-related hepatic failure: n = 13 HBeAg+ CHB in telbivudine longitudinal trial: n = 15					The expression levels of inhibitory markers in the CXCR5-FOXP3+ T population, including CTLA-4, were significantly upregulated after being stimulated by recombinant HBeAg and - HBcAg, but not HBsAg The frequency of CD4+CXCR5-FOXP3+ T cells was significantly lower in patients with a complete response** than in non-complete responders Substantial decrease in CTLA-4 expression at week 12 was observed in complete responders compared to non-complete responders.
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AHB = acute hepatitis B infection; ASC = asymptomatic carrier; CHB = chronic hepatitis B virus infection; CHB pre = pre-treatment; CHB post = post treatment with antiviral therapy; CMV = cytomegalovirus; HC = healthy controls; HCV = hepatitis C virus; HCC = hepatocellular carcinoma; HDV hepatitis D virus; human immunodeficiency virus = HIV; pre = pre-treatment; post = post-treatment; RHB = resolved hepatitis B infection.

*) Related to CTLA-4

**) i.e., HBeAg seroconversion and HBV DNA levels < 300 copies/ml

Table S4. Overview of included studies. Tregs

Source	Aim	Study participants (n)	Inclusion criteria	Exclusion criteria	Main T-cell type	Global – or HBV specific T cells	Primary findings
Park, J. J. [23] (2016)	To examine whether T-cell effector and regulatory responses can define clinical stages of CHB	CHB: n = 200 HC: n = 20	HBsAg-positive ≥ 18 years	Antiviral therapy HIV-infection HCC Hepatic decompensation Liver transplant Autoimmune disease Medications, or comorbid illnesses impacting immune response.	CD4+ Tregs and CD8+	HBV specific (+ Flu specific)	Upregulation of CTLA-4 on Tregs
Peng, G. [15] (2008)	To analyse the frequency and phenotype of Tregs in patients of different HBV infection status and investigate the effect of Tregs on antiviral immune responses in CHB patients.	CHB: n = 79 ASCs: n = 26 AHB: n = 12 HC: n = 20	HBsAg-positive	Antiviral therapy or steroids past 6 months	Tregs		No significant difference of the total Treg-frequency between groups. The frequency of Tregs was significantly increased in HBeAg+ CHB patients. In CHB patients, the frequency of Tregs positively correlated with viral load, and the Tregs could suppress the proliferation and IFN- γ production. Compared with CD25 ⁻ T-cells, CD25 ^{high} cells had significantly elevated expression of CTLA-4.
Stoop, J. N., [17] (2005)	To determine whether Tregs are	CHB: n = 50 RHB: n = 9	CHB patients	Antiviral or immuno-modulatory therapy past 6 months	Tregs		Patients with CHB have an increased proportion of Treg compared to HC.

	involved in the inadequate immune response leading to CHB.	HC: n = 23		HIV, HAV, HCV, HDV or other viral hepatitis infection Immunocompromised Pregnancy			No correlation between viral load or hepatic inflammation and the percentage of peripheral blood Treg Depletion of CD25+ cells from PBMCs of CHB patients resulted in an enhanced proliferation after stimulation with HBV core antigen.
Stoop, J.N., [27] (2008)	To examine the phenotype of FoxP3+ regulatory T cells in the liver of patients with CHB	CHB: n = 32 (7 samples used for detailed phenotypic analysis of Treg)	CHB patients	HAV, HCV, HDV or HIV infection Resolved viral hepatitis	Tregs		The liver contained a population of CD4+FoxP3+ cells that did not express CD25, while these cells were absent from peripheral blood. Intrahepatic CD25-FoxP3+CD4+ Tcells had lower expression of CTLA-4 and HLA-DR compared to their CD25+ counterparts. No correlation between ALT, metavir score and the proportion of Treg Patients with a high viral load have a higher proportion of Treg in the liver, but not in blood
Zhang, H. H [16] (2010)	To investigate frequency and phenotype of Tregs in CHB and HCC	CHB: n = 15 HCC: n = 49 HC: n = 25	CHB patients: CHB diagnosis	Antiviral therapy past 6 months Liver cirrhosis	Tregs	Global	CHB patients had a higher percentage of circulating and liver-resident Tregs compared to HC. In co-cultures, human hepatoma cell lines (HepG2 and HepG2.2.15) increased the expansion of Tregs – especially HepG2.2.15

							CTLA-4 was upregulated after being co-cultured with HepG2 and especially HepG2.2.15 cells
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AHB = acute hepatitis B infection; ASCs = asymptomatic HBV carriers; CHB = chronic hepatitis B virus infection; CMV = cytomegalovirus; HAV = hepatitis A virus; HC = healthy controls; HCC = hepatocellular carcinoma; HCV = hepatitis C virus; HDV hepatitis D virus; human immunodeficiency virus = HIV; RHB = resolved hepatitis B infection.