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Research paper

Sorted B cell transcriptomes point towards actively regulated B cell responses during ongoing chronic hepatitis B infections



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ABSTRACT

The natural course of chronic hepatitis B virus (HBV) infections follows distinct clinical disease phases, characterized by fluctuating levels of serum HBV DNA and ALT. The immune cells and their features that govern these clinical disease transitions remain unknown. In the current study, we performed RNA sequencing on purified B cells from blood ($n = 42$) and liver ($n = 10$) of healthy controls and chronic HBV patients. We found distinct gene expression profiles between healthy controls and chronic HBV patients, as evidenced by 190 differentially expressed genes (DEG), but also between the clinical phenotypes of a chronic HBV infection (17–110 DEG between each phase). Numerous immune pathways, including the B cell receptor pathway were upregulated in liver B cells when compared to peripheral B cells. Further investigation of the detected DEG suggested an activation of B cells during HBeAg seroconversion and an active regulation of B cell signalling in the liver.

1. Introduction

Increasing evidence suggests an important role for B cells in the pathogenesis of chronic hepatitis B virus (HBV) infections [1–5]. Treatment with B cell depleting agents such as the anti-CD20 monoclonal antibody rituximab is well known to reactivate HBV replication risking hepatic flares and fatal outcomes, even in patients with a resolved infection [6]. Peripheral blood B cells were shown to be phenotypically activated during a chronic HBV infection, with an increased expression of the surface markers CD69 and CD83 and antibody production, but a reduced proliferative capacity [7–9].

The natural course of a chronic HBV infection discerns four distinct clinical phases with alternating HBV load and Alanine Aminotransferase (ALT) levels: a HBeAg positive Immune Tolerant (IT) and Immune Active (IA) phase and a HBeAg negative Inactive Carrier (IC) and HBeAg

negative hepatitis (ENEG) phase [10]. Significant viral replication is observed in the IT, IA and ENEG, but not in the IC phase [10]. ALT elevations, indicating ongoing liver damage, are mostly seen in the IA and ENEG phases. As such, the length of both phases predisposes to rapid fibrosis progression [11]. In contrast, patients in the IC phase show an almost negligible risk of fibrosis progression [11].

The nature of the viral or immune mechanisms discriminating these clinical phases and their involvement in the transition between consecutive phases is a matter of intense research. Our group identified an immune gene signature consisting of many B cell related genes in whole blood and liver tissue of chronic HBV patients that correlates with the distinct clinical phases, and was found to be upregulated in the IA and ENEG phase [12,13]. In line with this, in a subsequent study, we found elevated levels of antibodies against the hepatitis B core antigen (HBcAg) in the IA and ENEG phase, whereas levels of antibodies against

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the hepatitis B surface antigen (HBsAg) did not significantly differ between clinical phases [4].

Using RNA sequencing, we previously uncovered important differences between sorted NK cells from viraemic patients and healthy controls [14]. The sorted NK cell transcriptome signatures differed substantially between viraemic HBV patients, HCV patients and HIV patients when compared to matched healthy controls, but barely any differences between HBV's clinical phases could be detected [14]. To further unravel the role of B cells during the different phases of an ongoing chronic HBV infection, we now use the same approach by performing RNA sequencing on purified B cells obtained from blood and liver of chronic HBV patients. This technique may uncover genes that are activated *in vivo* during chronic HBV infections, but that are not identified through biased phenotypic approaches.

2. Methods

2.1. Patients

Heparinized blood was obtained from 38 treatment naïve chronic HBV patients, and 13 unvaccinated healthy individuals (negative for HBsAg, anti-HBcAg, and anti-HBsAg). Blood samples of the healthy individuals were collected during outreach community screenings in the Chinese migrant population in Antwerp [15–17]. Blood from chronic HBV patients was sampled at the moment they underwent a liver biopsy for routine clinical care at the Antwerp University Hospital, ZNA Stuijvenberg (both located in Antwerp, Belgium) and the Erasmus Medical Center (Rotterdam, the Netherlands). When more liver tissue than strictly needed for routine histopathological diagnosis (upon discretion of the treating physician) was available, the liver biopsy was split into two: one part was fixed in formaldehyde for histological analysis, the other part was immersed in RPMI medium (ThermoFischer), supplemented with 10% fetal calf serum and 1% penicillin/streptomycin on ice. The latter fragment was further processed as described below.

Chronic HBV patients were excluded from the study when they showed (1) coinfection with the hepatitis C virus, hepatitis delta virus or HIV virus; (2) histological signs of non-alcoholic steatohepatitis; (3) histological liver fibrosis higher than F2 on the Metavir scale; (4) any concomitant auto-immune disease or (5) if they had received antiviral or immunosuppressive treatment less than 1 year before sampling. From one patient with a paired peripheral blood and liver sample, the peripheral blood sample showed haemolysis and was therefore excluded from further processing. All participants provided written informed consent.

Based on serum ALT levels, HBV DNA levels and HBeAg status chronic HBV patients were categorized into 4 clinical phases according to the guidelines proposed by the European Association of the Study of the Liver (EASL) (Table 1) [11,18–20]. For the sake of clarity we opted

to use the nomenclature as proposed by the American Association for the Study of the Liver, which was previously also used by the EASL [21]. IT patients had detectable HBeAg and no elevation of ALT levels above the upper limit of normal (ULN; defined as 40 U/L). IA and ENEG patients had repetitive or intermittent elevated ALT levels and HBV DNA > 2000 IU/mL within the year before sampling. IC patients had ALT levels < 1xULN and HBV DNA < 20,000 IU/mL. A substantial proportion of the HBeAg negative patients were classified as Grey Zone patients since they showed slightly elevated ALT levels in the absence of significant viral replication, or normal ALT with elevated viral replication and thus did not classify into the IC or ENEG phase. However, since their natural disease course closely mirrors that of the IC phase, Grey Zone and IC patients were pooled in the current study (Clinical courses depicted in Supplementary Fig. 1) [11]. The study was approved by the Institutional Review Boards of the participating centres and was performed in accordance with the guidelines of the declarations of Helsinki.

2.2. Cell sorting

Peripheral Blood Mononuclear cells (PBMC) were isolated from heparinized blood by Ficoll-Hypaque centrifugation and stored at -150°C . Liver biopsies were gently suspended with a plunger of a 1 mL syringe as described previously [22]. Liver cells were used fresh and stained as described below.

B cells were purified from PBMC and liver tissue based on CD19 positivity using a FACS Aria-II cell sorter equipped with a 100 μm nozzle and FACS DIVA software (BD Biosciences). Viable lymphocytes in PBMC were selected based on their FSC-SSC profile, and CD45 positive cells were selected in liver samples (Supplementary Fig. 2). Subsequently, 400–3000 intrahepatic and 3×10^3 – 4×10^6 peripheral CD3 negative, CD19 positive cells were sorted. The minimal sort purity of peripheral B cells was 90% (mean: $95.9 \pm 2.4\%$). Cells were washed twice and stored in RNeasy Protect[®] Cell Reagent (Qiagen) at -80°C until RNA isolation. Reagents used for flow cytometry were CD3 FITC (OKT3), CD19 PE (SJ25C1), and CD45 Pacific Blue (HI30), all from Biolegend; and CD3 PE (UCHT1), CD19 Pacific Blue (HIB19), and AlexaFluor 430 or AmCyan Live/dead, all from eBioscience/ThermoFisher.

2.3. RNA isolation and sequencing

Total RNA was isolated as per the manufacturer's instructions using the RNeasy micro kit (Qiagen) for samples with $>2 \times 10^5$ B cells or the Arcturus PicoPure RNA kit (ThermoFisher) for samples with a lower cell number. Initial quality check and RNA quantification was performed by capillary electrophoresis using the LabChip GX (Perkin Elmer). Non-degraded RNA samples were selected for sequencing at the University Medical Center Groningen, the Netherlands. Library preparation was performed using the Nextera XT RNA sample preparation kit (Illumina)

Table 1

Patient characteristics of samples obtained from liver and blood that were included in the analysis after strict quality control. Hepatic and peripheral samples were paired (same patient, same time point) in 4 IA patients and 5 IC/GZ patients. *: mean \pm SD.

	Healthy Controls (HC)	Immune Tolerant (IT)	Immune Active (IA)		Inactive Carrier/Grey Zone (IC/GZ)		HBeAg Negative Hepatitis (ENEG)
	Peripheral (n = 13)	Peripheral (n = 3)	Peripheral (n = 8)	Hepatic (n = 5)	Peripheral (n = 9)	Hepatic (n = 5)	Peripheral (n = 9)
Gender	7 males, 6 females	1 male, 2 females	5 males, 3 females	4 males, 1 female	6 males, 3 females	3 males, 2 females	8 males, 1 female
Ethnicity	13 Asians	3 Asians	5 Asians, 3 Africans	2 Asians, 2 Africans, 1 Caucasian	5 Asians, 2 Africans, 2 Caucasians	2 Asians, 2 Africans, 1 Caucasian	6 Asians, 2 Africans, 1 Caucasian
Age at sampling*	36 \pm 8	28 \pm 3	35 \pm 9	26 \pm 7	44 \pm 14	39 \pm 15	39 \pm 7
Histological fibrosis	NA	1 F0, 2 F1	2 F0, 3 F1, 3 F2	1 F0, 2 F1, 2 F2	6 F0, 3 F1	4 F0, 1 F1	3 F0, 4 F1, 2 F2
ALT (U/L) *	NA	20 \pm 8	96 \pm 62	73 \pm 44	35 \pm 8	34 \pm 12	56 \pm 17
HBV DNA (log IU/mL) *	NA	8.72 \pm 0.27	7.23 \pm 1.33	6.80 \pm 1.51	2.91 \pm 1.03	3.04 \pm 1.12	4.75 \pm 1.09

with a Sciclone NGS Liquid Handler (Perkin Elmer). In short, cDNA was generated, a pre-amplification Polymerase Chain (PCR) reaction was performed, adaptors including a Unique Molecular Identifier (UMI) barcode were annealed and the DNA fragments were amplified. In case of contamination by adaptor-duplexes an extra purification of the libraries was performed with the automated agarose gel separation system LabChip GT (PerkinElmer). The obtained cDNA fragment libraries were sequenced on an Illumina NextSeq500 using default parameters (paired-end read 2×75 bp) in pools of 6, equimolar samples.

2.4. Bio-informatic and statistical analyses

The generated FASTQ files were demultiplexed and quality trimmed with Trimmomatic using default settings [23]. Trimmed FASTQ files were then aligned to the human reference genome (v37, ensemble release 75) using STAR v2.5.1 with default settings and bam files sorted with picard v2.2.2 [24]. The Dropseq bio-informatics tool version 1.12 was used to demultiplex samples by UMI and to generate sample-specific gene read count tables [25]. First, samples with a total read count below 15,000 were removed in order to avoid noise in subsequent comparisons. Subsequently, genes with a mean read count per sample below 2 were regarded as potential noise and omitted from the final count table.

DEG were called using the DESeq2 package and were defined as genes with an absolute fold-change > 1.5 , and an adjusted p -value (Benjamini-Hochberg corrected) < 0.1 [26]. Four distinct DESeq2 transcriptome comparison approaches were applied: 1) all chronic hepatitis B patients ($n = 29$) versus healthy controls ($n = 13$); 2) all individual clinical phases (IT: $n = 3$, IA: $n = 8$, IC/GZ: $n = 9$ and ENEG: $n = 9$) versus healthy controls ($n = 13$); 3) Each individual clinical phase versus its subsequent: IT ($n = 3$) versus IA ($n = 8$), IA ($n = 8$) versus IC/GZ ($n = 9$), IC/GZ ($n = 9$) versus ENEG ($n = 9$); and 4) all intrahepatic samples ($n = 10$) versus all peripheral counterparts ($n = 17$). Pathway enrichment analysis was conducted among the detected DEG using the Ingenuity Pathway Analysis program (IPA; Qiagen). A pathway was defined as enriched when the calculated adjusted p -value for enrichment was < 0.05 . For each comparison, all pathway analysis results are included in [Supplementary Table 1](#) and the fraction of immunologically relevant pathways is highlighted in yellow. In a next approach, we focused on the individual DEG with known immune-related functions based on previously published data [14,27,28]. The R package 'Heatmap Plus' was used to create heatmaps based on DESeq2 normalized read counts. Cohort characteristics are either described (gender, ethnicity and fibrosis) or depicted as mean \pm standard deviation (ALT, age and HBV DNA).

3. Results

3.1. Cohort characteristics

A total of 38 chronic HBV patients and 13 healthy controls were included in this study. Peripheral B cells were purified from PBMC of all included subjects. After applying the strict quality criteria for sample integrity (cfr. Methods), peripheral B cell samples of 9 chronic HBV patients were excluded from further analyses, resulting in a total of 29 peripheral B cell samples from chronic HBV patients that were included in the final cohort: 3 were derived from IT patients, 8 from IA patients, 9 from IC/Grey Zone (IC/GZ) patients and 9 from ENEG patients. Paired liver tissue was analyzed from 4 IA and 5 IC/GZ patients from which also PBMC were available. Liver tissue was available from one additional IA patient whose peripheral blood sample showed severe hemolysis and was therefore discarded from further analysis. Baseline demographic and clinical phase related characteristics of the subjects included in downstream analyses are displayed in [Table 1](#), and a more detailed clinical characterization of the included patients is presented in [Supplementary Fig. 1](#) and [Supplementary Table 2](#). All chronic HBV patients were mono-infected, antiviral treatment naïve and had no signs of NASH

or significant fibrosis ($>F2$) at sampling. To account for potential inter-group differences in age, gender and ethnicity, these factors were corrected for when calling DEGs.

3.2. The transcriptome of peripheral B cells from chronic HBV patients is reflective of higher activity levels as compared to B cells from uninfected individuals

To determine the consequence on gene expression of HBV infection in peripheral B cells, we performed RNA sequencing on purified B cells from PBMC of 29 chronic HBV patients and 13 healthy individuals. Bio-informatical analysis of the data set identified transcripts of 11,544 genes. Of these, 190 were differentially expressed between diseased patients and healthy controls with a fold change of at least 1.5. IPA pathway analysis showed an enrichment for 12 pathways ([Supplementary Table 1](#)), among which 4 with a known immunology-related function, including the TREM1 signalling pathway, a pathway that influences Toll-Like Receptor pathways [29]. As presented in [Fig. 1A](#), 91 genes were upregulated, and 99 genes were downregulated in B cells derived from chronic hepatitis B patients versus healthy controls. Analysis of the expression of individual genes revealed genes linked to activation among the upregulated genes in chronic hepatitis B patients: CD83 (fold change 3.7) and CD69 (fold change 2.3) and the chemokine receptor CXCR4 (fold change 1.7). Among the downregulated genes are CD300A, CD200R1 and TNFAIP8L2 (fold change -5.5 , -5.0 and -4.7 , respectively), which have all been described to be involved in inhibitory signalling in immune cells, including B cells ([Fig. 1B](#), and [Supplementary Table 3](#) for a complete list of all DEG).

3.3. Clinical phases vs healthy individuals

To determine clinical phase specific transcriptome differences, we next compared the transcriptome of each separate clinical phase to healthy controls.

Pathway enrichment analysis among the different DEG comparisons ([Supplementary Table 1](#)) pointed to several immune related pathways when B cells derived from IA, IC/GZ and ENEG patients were compared to Healthy Controls, but not when B cells derived from IT patients were compared to HC. As presented in [Fig. 2](#), more detailed analysis of DEGs in each individual clinical phase versus healthy controls showed that the highest number of DEG was found for B cells obtained from IA and IC/GZ patients, and a lower number of DEG in IT and ENEG patients. Further exploration of the expression of individual genes showed that in B cells of IA patients, expression of CD83, CXCR4 and the activating gene CD300c as well as various ISG (APOBEC, RSAD2) were higher as compared to B cells from healthy individuals ([Fig. 2](#) and [Supplementary Table 4](#)). Also, in B cells from IC/GZ patients higher gene expression levels of activating genes were found; these include CD69, CD83, as well as CD160. Moreover, as presented in [Supplementary Table 4](#), among the upregulated DEG of B cells from IC/GZ and healthy individuals CXCR1, EOMES, KIR3DL2 and LAG3 were identified. Comparison of gene expression levels of B cells from ENEG patients with healthy individuals identified only few genes related to B cell activation, with a potential consequence of the downregulated expression of the inhibitory receptor CD300A in B cell activation.

Combined, our data suggest that gene expression profiles in blood B cells during chronic HBV phases which coincide with HBeAg seroconversion hint towards a more activated phenotype, whereas the transcriptome of B cells during the early (IT) and late (ENEG) stage of chronic HBV more resembles that observed in B cells from healthy individuals with less signs of activation of the B cell compartment.

3.4. Transition to subsequent clinical phase

Pronounced gene expression differences can be found when chronic HBV patients are compared with healthy uninfected controls, as patterns

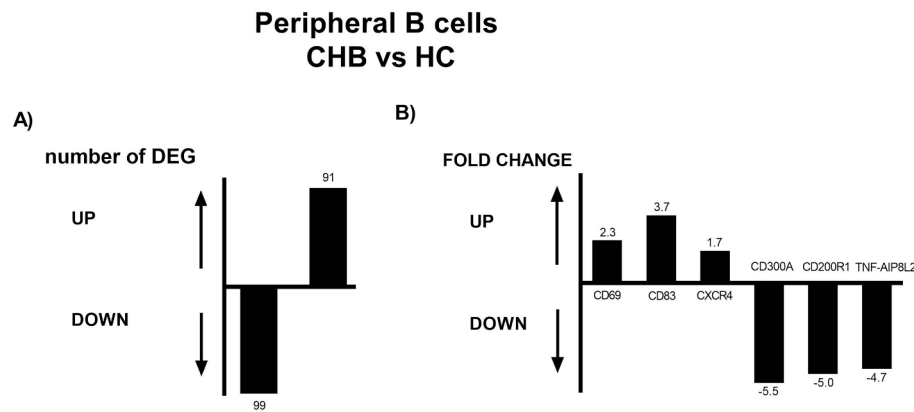


Fig. 1. Differential Gene Expression Analysis of peripheral B cells of all Chronic Hepatitis B patients versus Healthy Controls; A: number of differential expressed genes; B: Overview of a few immune relevant genes. CHB: Chronic Hepatitis B. HC: Healthy Controls.

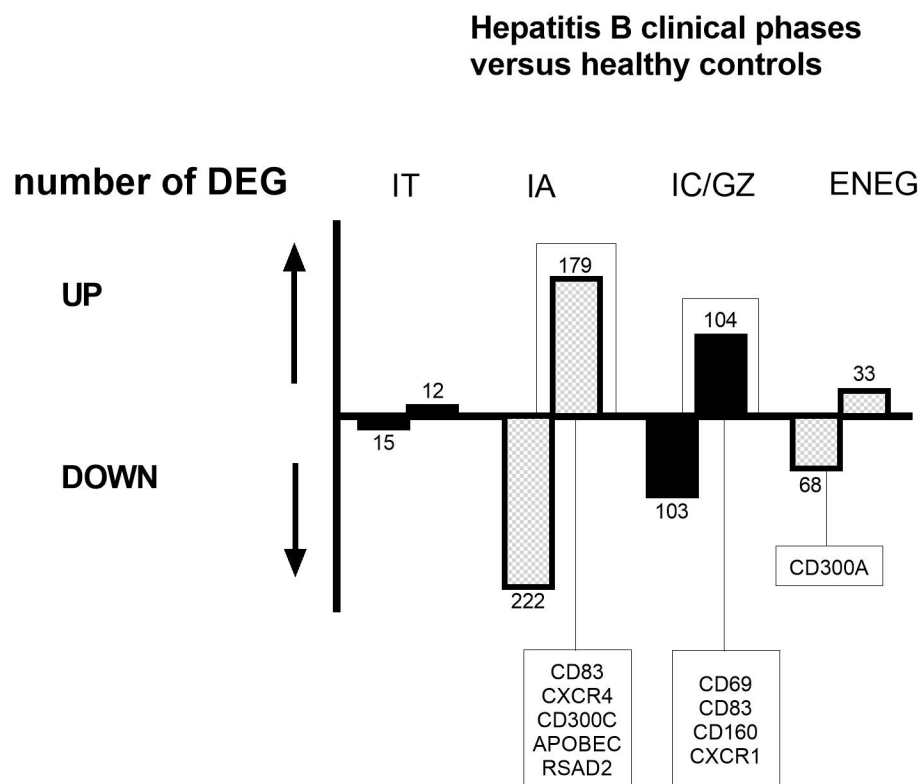


Fig. 2. Differential Gene Expression Analysis of each separate clinical Chronic Hepatitis B phase versus Healthy Controls. DEG: Differentially Expressed Genes. IT: Immune Tolerant. IA: Immune Active. IC/GZ: Inactive Carrier/Grey Zone. ENEG: Hepatitis B e Antigen Negative Hepatitis.

are determined by both the presence of HBV infection and liver disease. More subtle differences are to be expected when different phenotypes of the same disease are compared. To study B cell transcriptome changes in blood throughout the natural history of a chronic HBV infection, we compared the transcriptome of B cells from each clinical phase to that of the subsequent clinical phase (Fig. 3A). Strikingly, little overlap in DEG was found between the different comparisons (Fig. 3B).

Peripheral B cells isolated from IA phase patients showed a differential expression of only 17 genes (1 downregulated, 16 upregulated) when compared to peripheral B cells of IT phase patients. Pathway enrichment analysis (Supplementary Table 1) showed an enrichment for three immune related pathways due to a differential expression of the NME1 and the CSNK2B gene, and 13 non-immune related pathways. Further exploration of the individual genes showed that none of the differentially expressed genes encoded for proteins with specific

immune activity, but instead they encoded for transcriptional regulators and enzymes (Fig. 3 and Supplementary Table 5). Comparison of genes expressed by B cells from patients in the IA and IC phase identified a total of 78 DEG (42 downregulated in IA vs IC, 36 upregulated in IA vs IC) (Fig. 3A). Pathway enrichment analysis (Supplementary Table 1) pointed to several cell cycle and immune related genes. Among the genes expressed at lower levels in B cells from IA patients were THBS1 (encoding for thrombospondin 1) and the stimulatory receptor TNFRSF4 (encoding for the OX40 receptor), while the tyrosine kinase BTK, BCL2A1 and CD200R1 were expressed at higher levels in IA B cells (Fig. 3C).

Finally, comparison of gene expression profiles of blood B cells of IC/GZ phase patients with B cells of ENEG phase patients showed 110 differentially expressed genes (34 downregulated in IC, 76 upregulated in IC). Pathway analysis (Supplementary Table 1) showed an

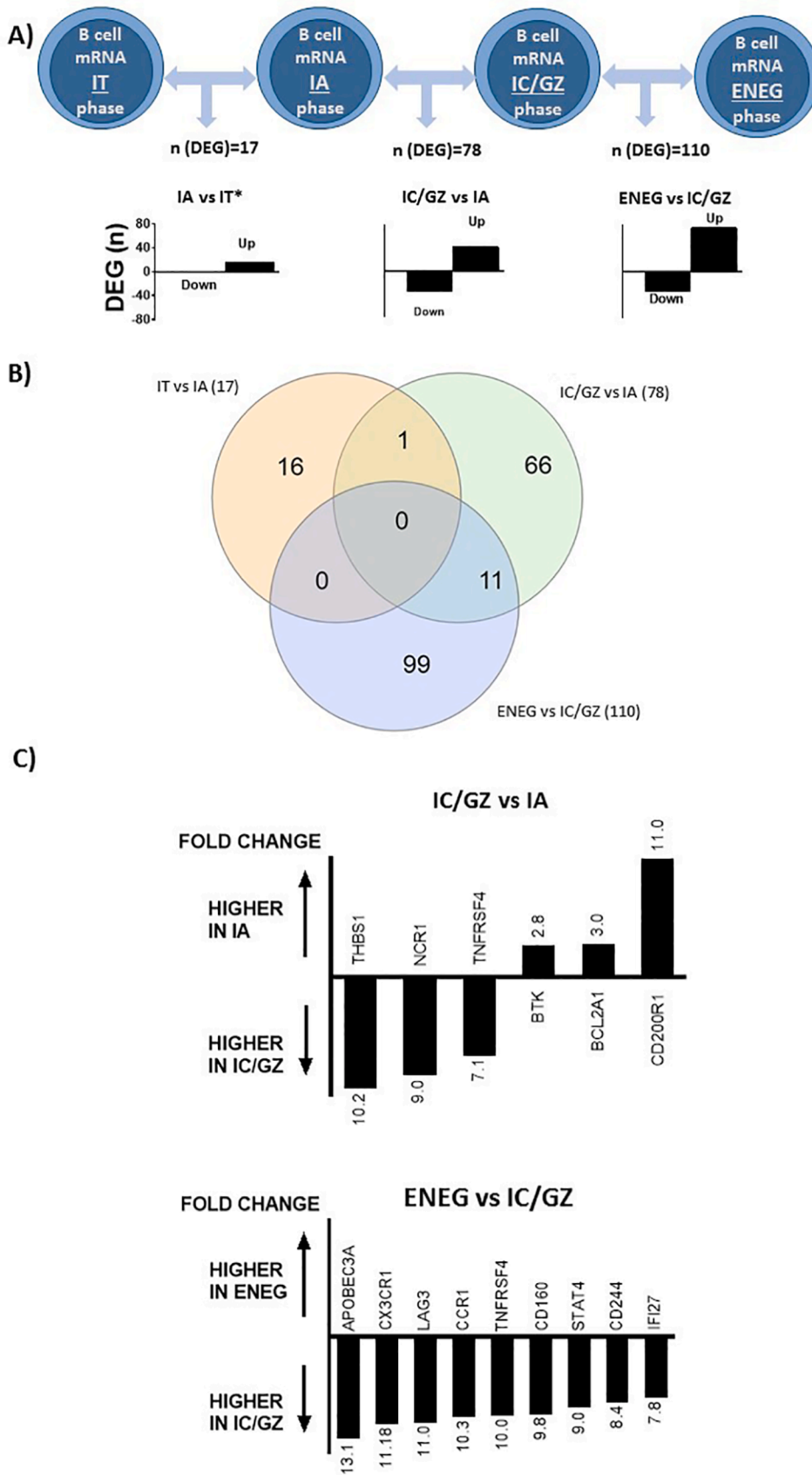


Fig. 3. Differential Gene Expression Analysis of each separate clinical phase versus its subsequent. A: Number of Differentially Expression Genes (DEG). B: Venn Diagram showing the overlap in DEG between different comparisons. C: Overview of selected immune related genes in the different comparisons. IT: Immune Tolerant. IA: Immune Active. IC/GZ: Inactive Carrier/Grey Zone. ENEG: Hepatitis B e Antigen Negative Hepatitis. *: comparison of the IA and IT phase did not show immune related genes (Supplementary Table S4).

enrichment for predominantly immune-related pathways (6 out of 19 enriched pathways). As presented in Fig. 3C and in Supplementary Table 5, exploration of the expression of individual genes expressed at higher levels in IC/GZ versus ENEG patients revealed a large number of important immune genes, including TNFRSF4, CD160, LAG3, CD244, STAT4, the chemokine receptors CXCR1 and CCR1, the ISG APOBEC3A and IFI27. Since the majority of the overlapping differentially expressed genes has higher expression in the IC phase as compared to the IA or especially the ENEG phase, it is tempting to speculate that B cell activity is more potent in the IC/GZ phase.

3.5. Intrahepatic B cell transcriptome profiling points to important differences between intrahepatic and peripheral B cell transcriptomes

As the liver is the site of infection and replication of HBV, we next explored the transcriptome profile of intrahepatic B cells isolated from liver biopsies of chronic patients. Overall, gene expression profiles of intrahepatic B cells revealed to be very different from their peripheral counterparts. Unsupervised clustering of paired liver and blood B cell samples (total $n = 18$) based on the normalized read count values of 8797 transcripts that were detected in both intrahepatic and peripheral B cells was able to distinguish liver from blood derived samples (Fig. 4A). The variation in expression levels was considerably higher in liver B cells than in peripheral blood derived B cells.

Analysis of all differentially expressed genes in all intrahepatic B cell samples ($n = 10$) versus peripheral B cells derived from all patients in the same phases (total $n = 17$; Table 1) showed that 703 genes were differentially expressed (Supplementary Table 6). Pathway analysis showed an enrichment for numerous pathways related to immune signaling (97 out of 341 identified pathways, Supplementary Table 1 and Supplementary Fig. 3), all pointing towards a transcriptionally more active status of intrahepatic compared to peripheral B cells obtained from chronic HBV patients.

Importantly, this was further supported by the exploration of the expression of the individual DEG. Overall, the expression of 638 DEG was found to be higher in the intrahepatic B cells as compared to peripheral B cells (Fig. 4B). Among the genes expressed at higher levels in liver B cells included activation-related genes CD69 and CD83 (fold change 5.0 and 3.2, respectively), chemokine receptors CCR1, CCR2, CC5, CCR7, CX3CR1, CXCR2 (fold change ranging from 2.9 to 16.9) and

also numerous ISG (e.g. IFI16, IFI27, IFI30, IFIT1, IFIT2) (Fig. 4C). These data clearly suggest that on the basis of their gene expression profile, B cells residing in the liver of chronic HBV patients display all signs of a more activated state than B cells from the periphery.

4. Discussion

In this study, we profiled the transcriptome of B cells derived from healthy controls and chronic hepatitis B patients in the different phases of a chronic HBV infection. A first comparison of chronic HBV patients versus healthy controls pointed towards a higher activation state of B cells in blood of the IA and IC/GZ phases, phases that coincide with HBeAg seroconversion. Blood B cells in both phases showed an upregulation of genes encoding the activation markers CD69 and CD83 and a downregulation of genes encoding inhibitory receptors. These findings are in line with previous whole blood transcriptome results from our group, where we showed that respectively 56% and 39% of DEGs were immunoglobulin related when the IA and IC phase were compared to the IT phase [12]. In our current study, signs of activation were not observed in sorted global B cells from the IT and ENEG phase, suggesting that the originally found whole blood immune signature for both phases is driven by other immune cell types or viral factors other than the here accounted for HBV viral load, such as the quantitative HBsAg levels, nucleocapsid or other viral related antigens [3,9]. Future mechanistic studies are needed to unravel the functional consequences of this transcriptional B cell activation. Interestingly, we found an upregulation of several Interferon stimulated genes in B cells derived from IA phase patients when compared to healthy controls, possibly pointing towards other functions for B cells than antibody production alone, as has also been suggested by another recent study in HBV specific B cells [3].

The investigation of B cell transcriptome changes in blood throughout the natural history of a chronic HBV infection further suggested clinical phase specific transcriptome profiles. Many DEGs were noted when each clinical phase was compared to its subsequent phase, but – more importantly- at least 80% of the detected DEGs were unique to one comparison (Fig. 3B). The finding that phase-specific B cells have a distinct gene expression profile is important, since phase-specific differences have not yet been convincingly described for T cells and NK cells. Indeed, although vigorous multi-specific T cell responses are crucial to obtain viral clearance, T cell responses to HBV do not correlate

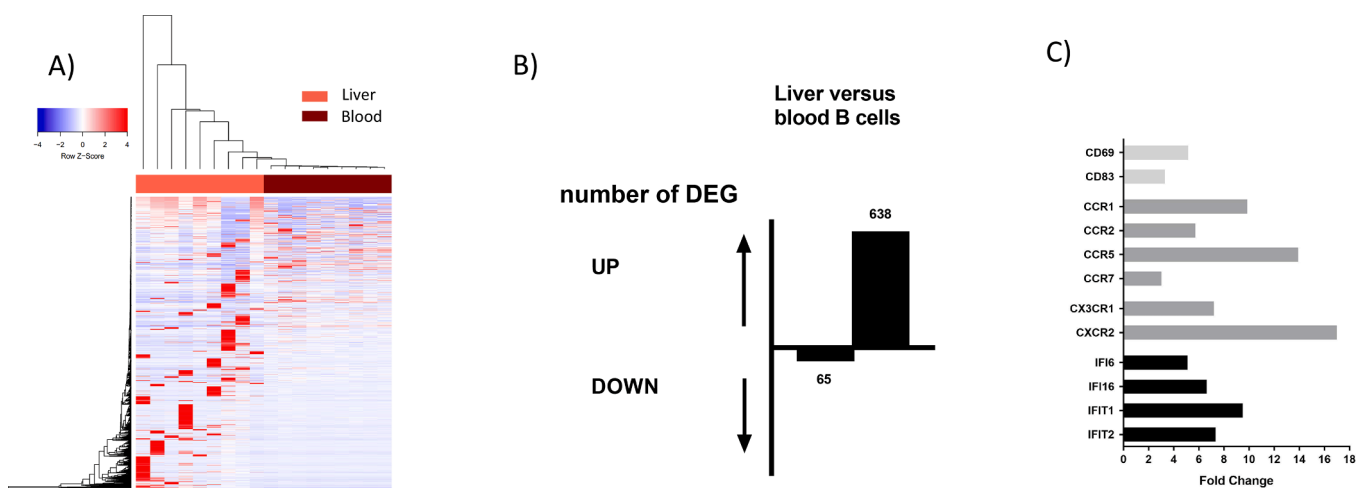


Fig. 4. Comparison of intrahepatic B cells versus peripheral B cells. A: Relative expression of all transcripts ($n = 8797$) that were detected in both liver and blood derived B cells of patients for whom a paired liver and blood sample was available. (hierarchical clustering based on the Euclidean distance between Z-scores). B: Number of Differentially Expressed Genes (DEG). C: Overview of immune relevant genes among the DEG that were higher expressed in liver vs peripheral blood B cells.

with the clinical phenotype of an ongoing chronic HBV infection [30,31]. Furthermore, in previous studies we were unable to define a clear NK cell signature that correlates with the different phases of a chronic HBV infection by transcriptomic and phenotypic characterization of NK cells [14,32].

In this study, we also showed that intrahepatic B cells display a distinct gene expression profile as compared to peripheral B cells. Interestingly, the transcriptome of intrahepatic B cells showed an important upregulation of many signaling, metabolic and functional pathways as compared to peripheral B cells. These differences suggest that the liver environment may affect the B cells via the interaction with signals from HBV-infected hepatocytes, but also other parenchymal or intrahepatic immune cells. Also, the interaction of intrahepatic B cells with cells within the unique tertiary follicular structures as observed in biopsies of some chronic HBV patients may explain the distinct gene expression profile of liver versus blood B cells. These findings are in line with our previous findings in NK and T cells [22]. Both cell types showed an upregulation of genes related to activation when liver samples were compared to peripheral blood samples [22]. Therefore, our results further substantiate the need to include the intrahepatic compartment in future immunopathogenic chronic HBV studies.

Our study has a few limitations. Firstly, we sorted bulk B cell populations and thus could not separately analyze HBV specific B cells or different B cell subsets [3,4,9,33]. However, studies on B cell-HBV interactions are very scarce. This study represents a first approach that calls for a further investigation of B cell subsets and HBV-specific B cells given the fact that we uncovered significant differences at bulk level. Follow-up studies on sorted single cells, subsets of B cells and/or virus specific B cells are needed. Secondly, there were differences in ethnicity between different groups in this study. However, we carefully corrected for these differences when performing DESeq2 analyses. As such, we believe that this difference did not bring any biases to our results. Thirdly, although our study is the largest to date describing B cell transcriptomes of chronic hepatitis B patients using an unbiased RNA sequencing approach, its sample size is limited, especially of the group of IT patients. Therefore, we opted to focus on patterns of gene expression (e.g. a combination of different activation markers and/or different pathways pointing towards B cell activation) instead of single genes. Given the biological relevance of the former, we believe that, despite its limited sample size, our study provides important pilot data that call for further investigation. In addition, our sample size is in line with a previous study in NK cells [14].

In conclusion, we were the first to profile the transcriptome of both peripheral and intrahepatic B cells during the natural disease course of a chronic HBV infection. Our results point to an activation of B cells during a chronic HBV infection and especially in clinical phases during which HBeAg seroconversion takes place. Compared to peripheral B cells, intrahepatic B cells showed an upregulation of various immune signaling pathways, including the B cell receptor pathway. All together our study corroborates an important role for B cells during ongoing chronic HBV infections and provides a broad platform that calls for extensive studies on B cell responses in ongoing chronic HBV infections.

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help in organizing the control group sample collection.

Author Contributions

SVH, ZG, and PvdV performed the experiments. SVH, AB, and TVW conceptualized the study. BC and SVH performed the bio-informatic analyses, KL and PM supervised the bio-informatic analyses; LV, SB, PM, SF and RdK provided patients material. SVH, AB and TVW drafted the manuscript. All authors read and approved the final version of the manuscript.

Declaration of Competing Interest

SF is consultant and/or lecturer for Astra Zeneca, Roche, Bayer, Eisai, BMS, BI, Genentech, Inventiva, Genfit, Intercept, Novartis, Novo Nordisk, Allergan, Abbvie, Gilead, NGMBio. TVW has participated in Advisory Committees or Review Panels for: Janssen Pharmaceuticals, Gilead Sciences, Abbvie, BMS, WL Gore. He has also received grant/research support from: Gilead Sciences, Roche, BMS and speaking and teaching support from: Gilead Sciences, BMS. AB has been in consulting or in advisory boards for Gilead Sciences and Bristol-Myers Squibb and has received research grants from Roche, Gilead Sciences, Fujirebio, and Janssen. SVH, BC, SB, ZG, PM, PvdV, RdK, LV, PM and KL have nothing to disclose.

Data availability statement

All count tables and metafiles used in this manuscript are available at the EMBL-EBI biostudies database (accession number: S-BSST578).

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.cellimm.2021.104283>.

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