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Research Article

High CD163 Expression on Classical Monocytes Is Associated with Immune Control of HBV Infection in Noncirrhotic Patients

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Background and Aims. The functional impairment of monocytes may contribute to the persistence of HBV infection. This study aims to assess monocyte subpopulations, monocyte expression of CD163, plasma sCD163, and sTWEAK in patients with chronic HBeAg-negative HBV infection at different phases of disease. *Methods*. Fifty-nine patients with CHB, 9 with a history of HBsAg/anti-HBs seroconversion, were enrolled. The control group consisted of 15 healthy volunteers. Subpopulations of peripheral blood monocytes were distinguished by CD14 and CD16. Membrane expression of CD163 was assessed by flow cytometry, plasma sCD163 concentration by ELISA, and sTWEAK by bead-based multiplexed immunoassay system. *Results*. CD163 expression was increased in classical and intermediate monocytes in CHB patients and those with HBsAg/anti-HBs seroconversion. CD163 expression on classical monocytes was associated with status of immune control and thus significant in HBV infection as compared to active hepatitis. Plasma sCD163 concentration was increased in CHB patients and those with HBsAg/anti-HBs seroconversion vs. the control group. Positive correlations between plasma sCD163 and ALT, as well as APRI, were observed. Plasma sTWEAK concentration was lower in CHB patients in comparison to patients with HBsAg/anti-HBs seroconversion. *Conclusions*. Exposure to HBV antigens alters monocyte subsets' frequencies and activation. The expression of CD163 on classical monocytes increased in parallel with improved immune control of the HBV infection. Patients who seroconverted HBsAg had the highest expression of CD163 on monocytes, which suggests involvement of monocytes in immune control of HBV infection. Persistent inflammation is accompanied by higher CD163 expression and sCD163 level and lower sTWEAK level.

1. Introduction

Chronic hepatitis B virus (HBV) infection is accompanied by the impairment of immune system function leading to enhanced viral replication and progression of liver disease. Importantly, it is not yet fully understood why some chronically infected patients are able to control HBV replication, while in most infected subjects, the virus escapes immune surveillance. The dynamics of disease is especially pronounced in HBeAg(-) hepatitis, where fluctuations of HBV-DNA and inflammatory activity are a well-known phenomenon. In some patients, long-lasting immune control of viral replication can be naturally acquired, defined as HBeAg(-) infection. Those patients exhibit a favourable prognosis with low risk of disease progression. It has been confirmed that subjects achieving sustained immune control have the highest chances of HBsAg loss and seroconversion [1, 2]. Activation of the endogenous interferon system, multifunctional

HBV-specific CD4 T-cells, overcoming T-cell exhaustion, and innate immunity-associated factors are suggested to play a role in HBV viral load (VL) control [3, 4]. Understanding of the immune HBV infection control mechanism might allow for optimization of current treatment approaches and design of novel immunotherapies.

Monocytes represent a heterogenic population of immune cells, which, according to differences in CD14 and CD16 expression, can be divided into three functionally different subsets, namely classical (CD14++CD16-), intermediate (CD14++CD16+), and nonclassical (CD14+CD16++) monocytes. In physiological conditions, all three subsets, respectively, represent successive stages of monocyte maturation [5, 6]. Classical CD14++CD16- monocytes constitute the predominant subset of blood monocytes (80-90%). These cells are considered nonactivated cells with highly phagocytic activity. Upon stimulation, classical monocytes acquire CD16 marker expression and secrete a wide array of cytokines and chemokines. In physiological conditions, intermediate CD14++CD16+ monocytes comprise approximately 5% of total circulating monocytes and represent the transitional state of maturation between classical and nonclassical cells. Intermediate monocytes may serve as M2 macrophage precursors with high anti-inflammatory properties following LPS stimulation, producing high amounts of IL-10 [7–9]. M2-type macrophages were shown to exhibit tissue repair properties; however, it is not fully elucidated whether they can drive or contribute to the resolution of liver inflammation and fibrosis [10]. There is evidence for elevated numbers of intermediate monocytes in end-stage liver disease, where they were shown to modulate liver fibrogenesis [11, 12]. Nonclassical CD14+CD16++ cells represent the most mature subset, constituting around 10% of total circulating monocytes. They display significant proinflammatory properties, secreting high amounts of TNF and IL-1β in response to simulation [7]. Elevated numbers of nonclassical monocytes were observed in various inflammatory conditions [13–15]. It was reported formerly that the functional impairment of monocytes may contribute to the persistence of HBV infection. Importantly, monocytes are recognised as the only antigen-presenting cells that can internalize and store highly immunogenic viral antigens [16]. Numerous studies have demonstrated that the expression of pattern recognition receptors on monocytes is reduced in HBV-infected individuals and thus influences the initiation of antiviral immune responses and cytokine secretion [17, 18].

CD163 is considered a phenotypic marker of monocytes with anti-inflammatory potential and a differentiation marker of monocytes/macrophages, which functions predominantly as a scavenger receptor for the haemoglobin-haptoglobin complexes and tumour necrosis factor (TNF)-like weak inducer of apoptosis (TWEAK). Furthermore, CD163 is involved in binding/recognising bacterial and viral antigens. In general, cellular expression of CD163 is upregulated by anti-inflammatory factors, whereas proinflammatory signals downregulate its expression. Soluble CD163 (sCD163) is present in blood serum as a result of shedding the CD163 membrane form of activated monocyte-macrophage-lineage cells in the course of inflammation [19]. As liver Kupffer cells account for about 80% of macrophages in the body, serum level of sCD163 was proposed to serve as a new biomarker of liver necroinflammation and fibrosis [19, 20].

TWEAK as member of a TNF superfamily regulates the inflammatory response, proliferation status, differentiation, and cellular survival. TWEAK is a widely expressed membrane anchored receptor, but also has a cleaved soluble form (sTWEAK). The latest evidence suggests that TWEAK may be involved in regeneration and fibrogenesis of the liver through proliferation activation in liver progenitor cells and hepatic stellate cells [21, 22]. Interestingly, the CD163 molecule might function as a scavenger receptor for sTWEAK, thus modulating inflammatory responses [23].

At the moment, there are scarce data on monocyte expression of CD163, serum levels of sCD163, and sTWEAK in chronic HBV infection. Therefore, in the current study, we set out to analyse for the first time the mutual relationships among monocyte subpopulations, monocyte CD163 expression, plasma sCD163, and sTWEAK in various phases of HBV infection including the immune control phase.

2. Material and Methods

2.1. Studied Population. Fifty-nine patients with chronic HBeAg-negative HBV infection (CHB), 6 with spontaneously resolved HBV infection with seroconversion HBsAg/anti-HBs ≥20 years earlier (RES) and 3 with treatment-associated seroconversion HBsAg/anti-HBs ≥2 years earlier (S-CONV), were enrolled in the study. The long-term follow-up (>36 months) in CHB patients allowed three patterns of chronic infection to be distinguished according to current EASL recommendations, in the same manner as it was done in our previous study [24, 25].

(i) HBeAg-negative chronic infection (ENI, $n = 17$)—HBV − DNA < 2000 U/L, normal aminotransferases

(ii) HBeAg-negative chronic hepatitis B naïve-to-treatment (ENH, $n = 6$)—HBV − DNA > 2000 U/L and elevated aminotransferases

(iii) ENH during nucleos(t)ide analogue therapy (tenofovir $n = 7$, entecavir $n = 6$, adefovir $n = 2$, or lamivudine $n = 2$, at the time of sample collection) with complete HBV-DNA suppression > 24 months (SUPR, $n = 17$).

Two additional groups were distinguished based on the HBV-DNA load and aminotransferases level:

(i) low-replicative hepatitis (ENH-LR) ($n = 3$)—HBV − DNA < 2000 U/L, elevated aminotransferases

(ii) high-replicative patients with HBV − DNA > 2000 U/L, normal aminotransferases (ENI-HR) ($n = 16$)

The characteristics of the studied groups are shown in Table 1. The control group (HC) consisted of 15 healthy volunteers. Blood samples were collected from fasting individuals without clinically evident acute inflammatory infection.

Table 1: Demographic, clinical, and laboratory characteristics of studied CHB groups. N/A: nonapplicable.

	HBV patients	HBsAg/anti-HBs seroconverted	Healthy subjects
Epidemiology			
Patients	60	9	15
Gender, m/f (%)	35/25 (58.3/41.7)	6/3 (66.7/33.3)	7/8 (46.7/53.3)
Age, median, range	37, 20-84	57, 23-79	38, 29-61
Laboratory results			
ALT (U/L), median, range	26, 11-100	22, 10-42	26, 11-43
AST (U/L), median, range	21, 8-53	N/A	N/A
CRP (mg/L), median, range (ULN = 5 mg/L)	0.8, 0.1-4.0	1.3, 0.6-1.8	0.6, 0.1-1.8
WBC (/nL), median, range	5.62, 3.36-9.0	6.36, 4.48-8.67	6.05, 4.05-7.21
Monocytes (%), median, range	10.0, 6.4-18.5	10.7, 8.5-17.9	9.4, 7.1-11.7
Neutrophils (%), median, range	54.1, 40.7-70.0	53.3, 10.1-65.4	51.8, 43.7-66.6
Lymphocytes (%), median, range	32.0, 18.6-44.3	34.8, 23.2-37.6	34.1, 24.5-42.2
Thrombocytes (/nL), median, range	209, 40-401	192, 101-272	233, 178-306
AST to platelet ratio index (APRI)	0.25, 0.09-1.0	N/A	N/A
HBV-DNA (log10 IU/mL), median, range	2.57, 1.28-7.64	N/A	N/A
qHBsAg (log10 IU/mL), median, range	4.06, 0.95-4.69	N/A	N/A
HBV genotype			
A	13 (72.2)	N/A	N/A
A+D	1 (0.6)	N/A	N/A
D	2 (1.2)	N/A	N/A
F	1 (0.6)	N/A	N/A
H	1 (0.6)	N/A	N/A
Histology—necroinflammatory activity grade (Scheuer), $n = 28$			
1, n (%)	8 (28.6)	N/A	N/A
2, n (%)	13 (46.4)	N/A	N/A
3, n (%)	7 (25.0)	N/A	N/A
4, n (%)	0	N/A	N/A
Histology—fibrosis stage (Scheuer)			
1, n (%)	21 (75.0)	N/A	N/A
2, n (%)	7 (25.0)	N/A	N/A
3, n (%)	0	N/A	N/A
4, n (%)	0	N/A	N/A

Informed consent was obtained from all individuals included in the study.

The exclusion criteria for the study included HCV, HIV, HAV infections, alcoholic liver disease, nonalcoholic steatohepatitis, autoimmune liver diseases, drug-induced liver injury, extrahepatic chronic inflammatory diseases, acute inflammatory conditions of any aetiology, and malignancies.

The study was approved by the institutional bioethical committee (no. R-I-002/497/2014). All procedures performed in the study were in accordance with the ethical standards of the institutional and national research committee and with the 1964 Declaration of Helsinki and its later amendments or comparable ethical standards.

2.2. Blood Collection and Flow Cytometry. 100 μL of fresh EDTA-anticoagulated whole blood was immediately stained using a panel of fluorescence labelled antibodies: CD14 PerCP, CD16 FITC, HLA-DR APC, CD163 PE (BD Bioscience, information on the antibodies used included in Table 2), according to the stain-and-then-lyse-and-wash protocol, as previously described [26]. Fluorescence-minus-one (FMO) controls were used for setting compensation and to assure correct gating. Specimen acquisition was performed

Table 2: Description of monoclonal antibodies used in the study.

Cell surface marker	Fluorochrome	Antibody clone	Company
CD14	PerCP	MφP9	BD Bioscience
CD16	FITC	3G8	BD Bioscience
CD163	PE	GHI/61	BD Bioscience
HLA-DR	APC	TÜ36	BD Bioscience

using a FACSCalibur flow cytometer (Becton Dickinson Bioscience). Overall number of acquired monocyte events constituted over 250×10^5 events for each sample. Obtained data were analysed using FlowJo ver. 7.6.5 software (Tree Star). The gating strategy for delineation of monocyte subsets is shown in Figure 1. Monocyte subpopulations were presented as frequencies within total pool of CD14-positive monocytes. Three monocyte subpopulations were distinguished on the basis of differential expression of CD14 and CD16. Expression of CD163 was analysed both as mean fluorescence intensity (MFI) on gated monocyte subpopulations and as frequency of CD163-postive cells in selected populations.

2.3. Immunoassays. sCD163 levels in EDTA-plasma samples were quantified by means of commercially available sCD163 DuoSet ELISA kit (R&D Systems), according to the manufacturer's instructions (range: 156 pg/mL-10 ng/mL).

Plasma sTWEAK concentrations were quantified using Luminex xMap technology (Bio-Plex 200 System, Bio-Rad, USA)—a bead-based multiplexed immunoassay system in a microplate format (limit of detection [LOD]: 0.5 pg/mL; assay working range: 3.1–6772.8 pg/mL); cat. no 171-AL001M, Bio-Rad, USA).

2.4. Statistical Analysis. The results were expressed as medians and range, unless indicated. The following nonparametric tests were applied: Mann-Whitney U test and Kruskal-Wallis ANOVA test for univariate comparisons and Spearman rank test for correlation analysis. Statistically significant p values were <0.05. Statistica 12 for Windows was used to perform the analysis (StatSoft Inc., Tulsa, USA), and graphical presentation of the results was performed using GraphPad Prism software (GraphPad Prism Software Inc., San Diego, CA, USA).

3. Results

3.1. Frequencies of CD14++CD16-, CD14++CD16+, and CD14+CD16++ Monocytes in Chronic HBV Infection. To examine whether HBV infection may have an effect on monocyte composition, we assessed the frequencies of three monocyte subpopulations. We observed that classical monocytes were less frequent in CHB compared to the HC and patients with a history of spontaneous or drug-induced HBsAg/anti-HBs seroconversion (S-CONV+RES groups). The percentage of intermediate monocytes did not differ between CHB, HC, and S-CONV+RES groups, while nonclassical monocytes were significantly increased in patients with CHB compared to the HC (Figure 1(c)).

3.2. Expression of CD163 on Peripheral Blood Monocytes in Different Phases of Chronic HBV Infection. We found significantly increased CD163 expression in the population of classical monocytes in patients with CHB and S-CONV+RES group vs. the HC group (Figure 2(a)). In CHB patients, expression of CD163 on classical monocytes was significantly higher in the ENI group than the ENH group (Figures 2(b) and 2(c)). Interestingly, in comparative analysis of CD163 expression on classical monocytes between all the groups distinguished in this study, we observed the lowest CD163 expression in the HC. Among groups with a history of HBV infection (CHB and S-CONV+RES), the highest CD163 expression was in patients with resolved HBV infection with seroconversion HBsAg/anti-HBs (S-CONV+RES) and in low-replicative phase ENI, lowest in patients with ENH (ANOVA $p = 0.02$ for frequency, $p = 0.03$ for MFI). Summing up, the expression of CD163 on classical monocytes increased in parallel with improved immune control of the HBV infection (ENH<ENI-HR<ENH-LR<SUPR<ENI≤S-CONV+RES).

CD163 expression on intermediate monocytes was significantly increased in patients with CHB and in S-CONV +RES groups in comparison to the HC (Figure 3(a)); however, we did not observe differences in CD163 expression across CHB groups (Figures 3(b) and 3(c)). There were no significant differences in CD163 expression on nonclassical monocytes between CHB, S-CONV+RES, and HC groups as well as across CHB groups (Figures 4(a)–4(c)). The lowest expression of CD163 was found on the nonclassical monocytes in all groups when compared to classical and intermediate monocytes (t-test, $p < 0.0001$), while the expression of CD163 on classical and intermediate monocytes was comparable (data not shown).

3.3. Plasma sCD163 and sTWEAK Concentrations in Different Phases of Activity of Chronic HBV Infection. Plasma sCD163 concentration was found to be significantly increased in patients with CHB and in S-CONV+RES group in comparison to the HC ($p = 0.0006$ and $p = 0.01$, respectively) (Figure 5(a)). Among CHB patients, we did not observe associations of sCD163 concentration and phase of HBV infection (data not shown). There was a negative correlation between plasma sCD163 concentration and mean intensity of fluorescence (MFI) of CD163 on classical monocytes. No significant associations were found between plasma sCD163 concentration and CD163 expression (MFI) on intermediate and nonclassical monocytes (Table 2).

Plasma sTWEAK concentration was significantly lower in CHB patients in comparison to S-CONV+RES group (Figure 5(b)). We did not find any specific associations between plasma sTWEAK and activity of HBV infection within the CHB group (data not shown). We found that plasma sTWEAK was negatively associated with the MFI of CD163 on each analysed subset of monocytes (Table 3).

3.4. Correlation of Viral (VL, qHBsAg) and Biochemical Parameters (ALT, APRI) with CD163 Expression on Monocytes, sCD163 and sTWEAK. To test whether CD163 expression on monocytes and plasma sCD163 and sTWEAK reflect the activity of HBV infection, the viral factors (HBV-VL, qHBsAg) and biochemical parameters were compared to monocyte CD163 expression, sCD163, and sTWEAK levels. We found a significant negative correlation between CD163 expression on classical monocytes and serum alanine aminotransferase (ALT) levels as well as APRI score (Table 4). Similarly, lower CD163 expression on intermediate monocytes was associated with increased ALT levels. Plasma sCD163 levels were significantly positively associated with

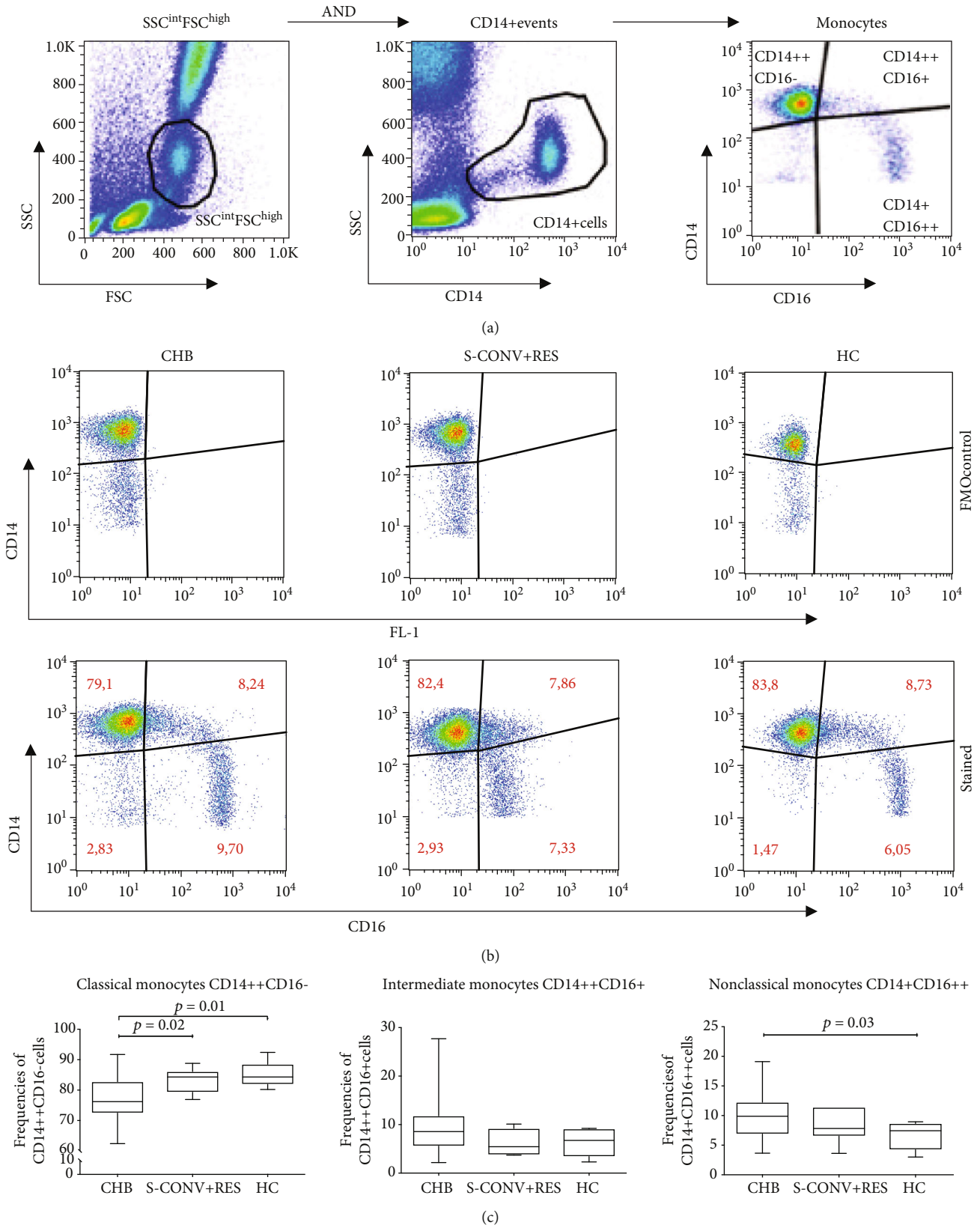

Figure 1: Peripheral monocyte frequencies in studied groups. (a) Gating strategy. (b) FMO controls for all studied groups (upper row) with representative dot plots of monocyte subsets in CHB, S-CONV+RES groups and healthy controls. Values in quadrants indicate the proportion of monocyte subset. (c) Summarised comparative analysis of monocyte subsets in CHB, S-CONV+RES patients, and healthy controls. p values are shown if significant. Horizontal bars represent median proportion of monocyte subsets.

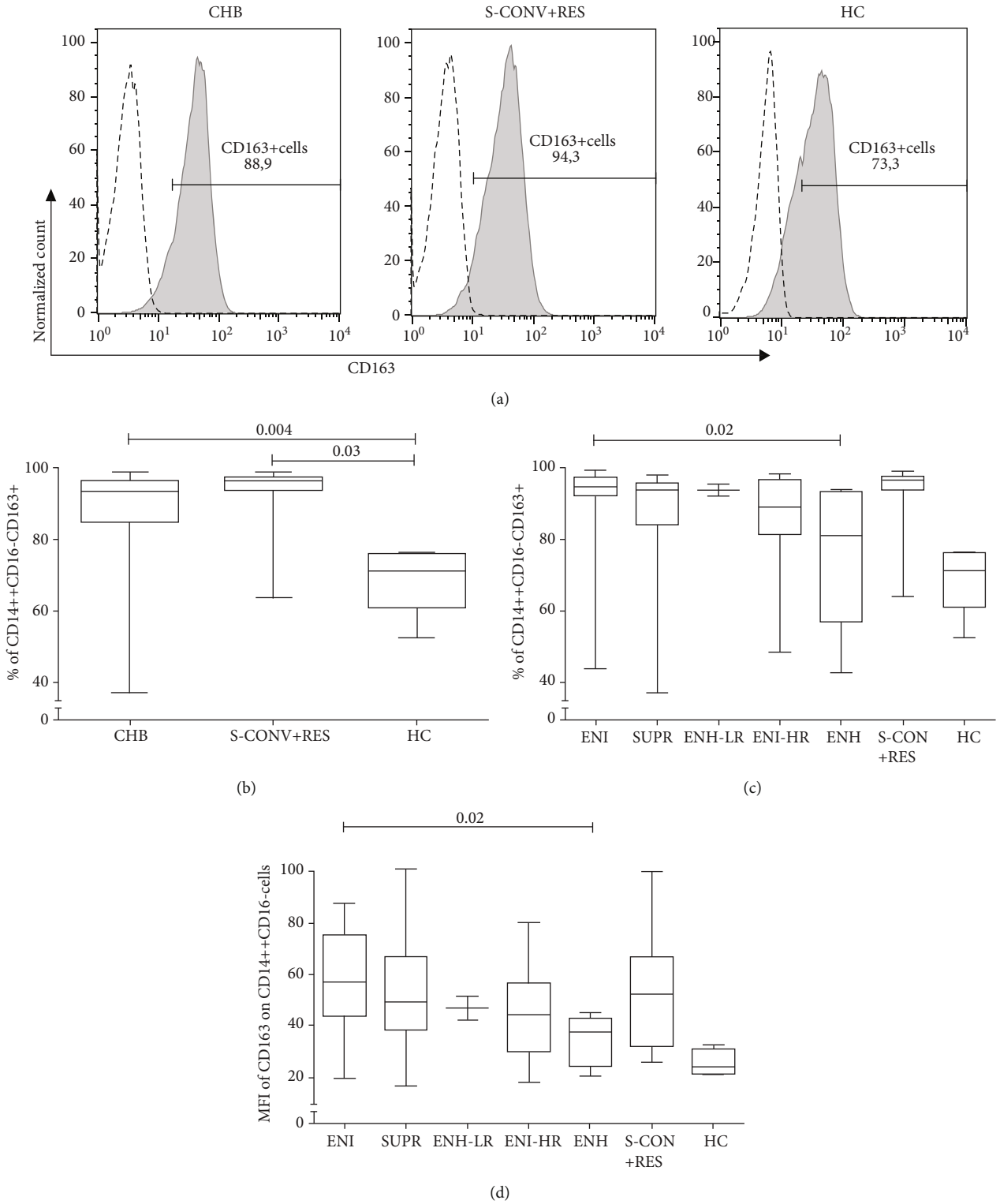

Figure 2: Frequency and expression of CD163 within classical monocytes. (a) Representative histograms showing CD163 expression in CHB, S-CONV+RES, and HC groups (solid lines), with adequate FMO controls (dashed lines). (b) Comparative analysis of CD163 expression on classical monocytes in CHB, S-CONV+RES, and HC groups. (c, d) Comparative analysis of CD163 expression on classical monocytes with reference to the phase of CHB infection. Data presented as median values with 25th and 75th percentile and minimum-maximum values.

serum ALT levels and APRI score. In line with this, there were trends towards lower CD163 expression on classical and intermediate monocytes as well as higher sCD163 and sTWEAK concentrations in patients with portal/periportal necroinflammatory activity in liver tissue ($G \geq 2$) in comparison to discrete portal inflammation ($G = 1$) ($p = 0.074$,

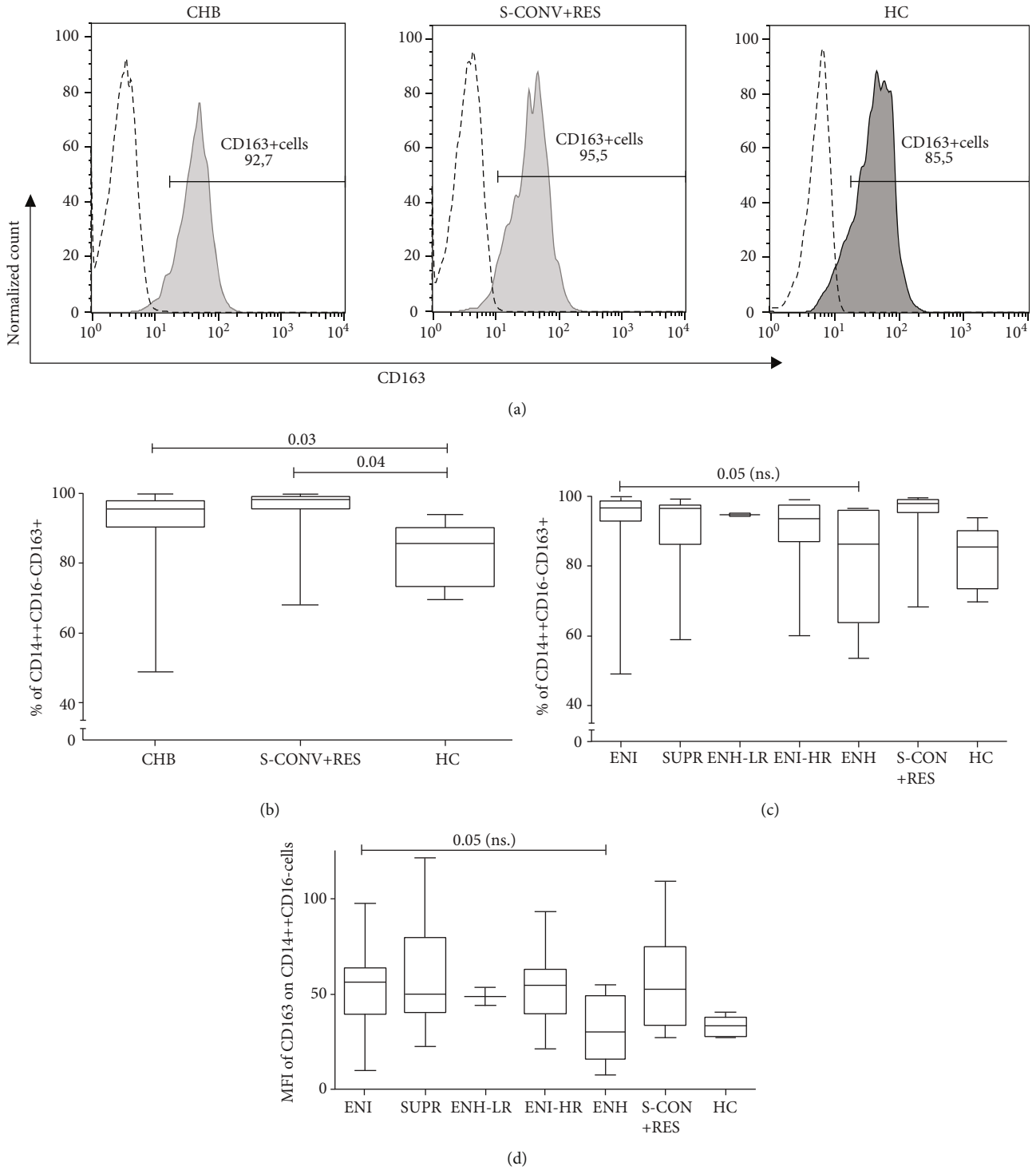

Figure 3: Frequency and expression of CD163 within intermediate monocytes. (a) Representative histograms showing CD163 expression in CHB, S-CONV+RES, and HC groups (solid lines), with adequate FMO controls (dashed lines). (b) CD163 expression on intermediate monocytes in CHB, S-CONV+RES, and HC groups. (c, d) Comparative analysis of CD163 expression on intermediate monocytes with reference to the phase of CHB infection. Data presented as median values with 25th and 75th percentile and minimum-maximum values.

$p = 0.0592$, $p = 0.057$, $p = 0.063$, respectively). No significant correlations were found between viral factors (HBV-VL, qHBsAg) and monocyte CD163 expression, plasma sCD163, or plasma sTWEAK.

4. Discussion

Currently, the primary objective in the management of HBV infection is to achieve the highest immune control of the

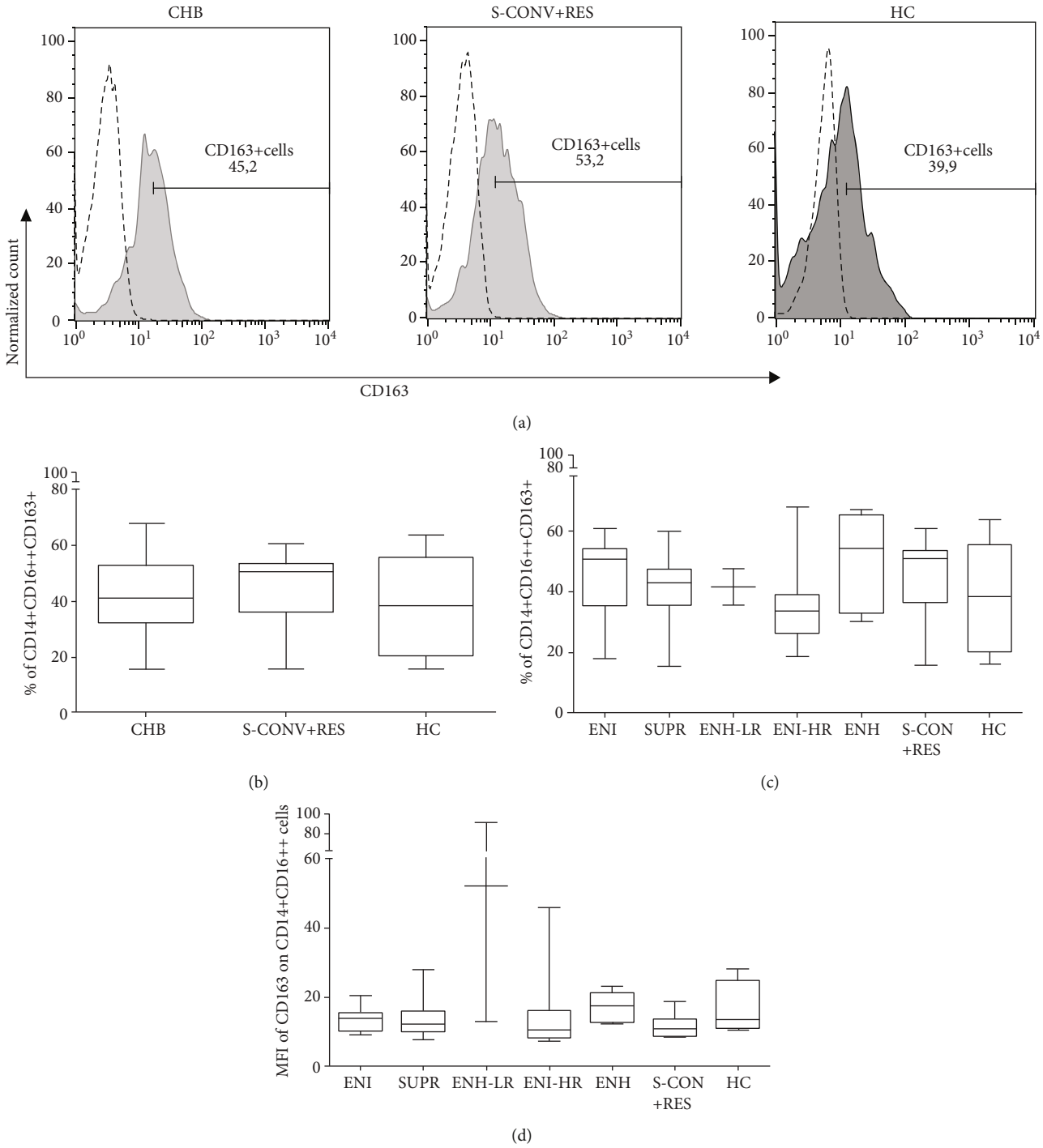

Figure 4: Frequency and expression of CD163 within nonclassical monocytes. (a) Representative histograms showing CD163 expression in CHB, S-CONV+RES, and HC groups (solid lines), with adequate FMO controls (dashed lines). (b) CD163 expression on nonclassical monocytes in CHB, S-CONV+RES, and HC groups. (c, d) Comparative analysis of CD163 expression on nonclassical monocytes with reference to the phase of CHB infection. Data presented as median values with 25th and 75th percentile and minimum-maximum values.

virus expressed as a HBsAg/anti-HBs seroconversion. Recently, there have been a few studies highlighting the important role of the innate immune response in the pathomechanism of HBV infection. In the search of immune targets allowing control over HBV to be restored, these reports are of great importance as innate immunity mechanisms might potentially be a basis for the development of novel therapeutic approaches [27].

Data presented in our study are the first evaluating associations between frequencies of monocyte subsets, monocyte expression of CD163, plasma sCD163, plasma sTWEAK, and chronic inflammation in the course of HBV infection. We

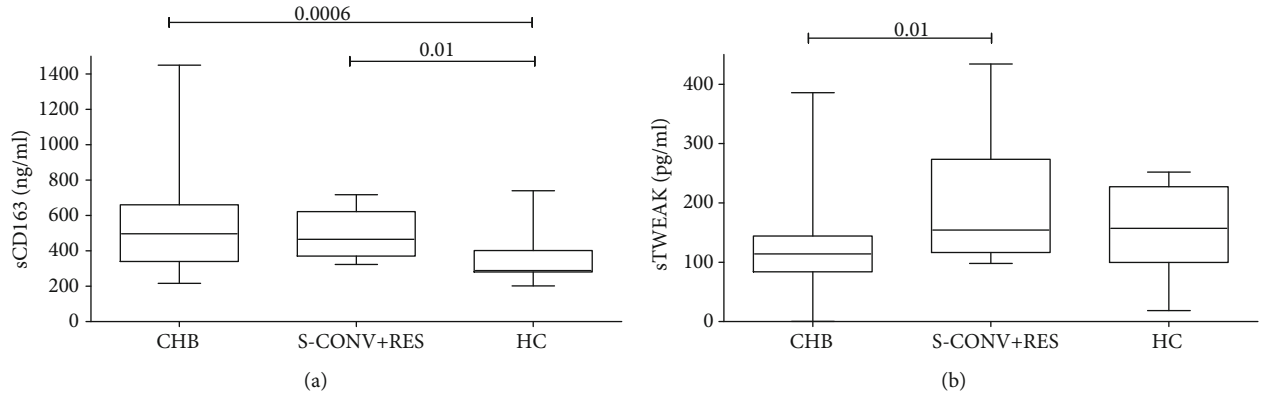

Figure 5: sCD163 and sTWEAK levels in plasma. (a) Plasma concentration of sCD163 in CHB, S-CONV+RES, and HC groups. (b) Plasma concentration of sTWEAK in CHB, S-CONV+RES, and HC groups. Data presented as median values with 25th and 75th percentile and minimum-maximum values.

Table 3: Correlations between sCD163/sTWEAK and CD163 expression (MFI) on monocyte subpopulations.

Correlations	sCD163 (ng/mL) *r* *p*	sTWEAK (pg/mL) *r* *p*
MFI of CD163+ on CD14++CD16- cells	**-0.281** **0.030**	**-0.353** **0.007**
MFI of CD163+ on CD14++CD16+ cells	-0.204 0.118	**-0.406** **0.002**
MFI of CD163+ on CD14+CD16++ cells	-0.166 0.210	**-0.291** **0.028**

found that unlike significantly reduced classical monocytes, nonclassical monocytes were the more dominating population in HBsAg-positive patients, in comparison to the control group. Moreover, CD163 expression was significantly increased in the population of classical and intermediate monocytes in CHB patients and those with HBsAg/anti-HBs seroconversion. Interestingly, CD163 expression on classical monocytes was associated with the status of immune control and thus significant in HBV infection (ENI) as compared to active hepatitis (ENH). The expression of CD163 on nonclassical monocytes was the lowest, presumably suggesting their less important role in HBV infection. Plasma sCD163 concentration was significantly increased in patients with CHB and S-CONV+RES group vs. the HC. Moreover, it correlated negatively with CD163 expression on classical monocytes. There was a positive correlation between plasma sCD163 and serum ALT, as well as APRI score. Furthermore, we observed that plasma sTWEAK concentration was significantly lower in CHB patients in comparison to S-CONV +RES group.

There are conflicting data on the influence of HBV infection on phenotype and activity of circulating monocytes. Boltjes et al. observed that both monocytes from healthy subjects and HBV-infected individuals produced cytokines upon exposure to HBsAg. Moreover, they did not observe significant differences in the *in vitro* responsiveness to HBsAg stimulation and ratios of monocyte subpopulations obtained from the patients at different phases of infection. However, at that time their analysis was based on the old approach to monocyte delineation, distinguishing only CD14++CD16- and CD14+CD16+ populations [28]. In contrast, Zhang et al. reported in HBeAg-positive patients increased ratios of CD16+ monocytes, both intermediate and nonclassical, with concomitant reduction of classical monocytes. This difference was particularly pronounced when the active HBeAg-positive hepatitis group was compared to healthy controls [29]. These findings are in agreement with our observations in the HBeAg-negative population. We observed a lower ratio of classical monocytes and higher ratio of nonclassical monocytes in the CHB group in comparison with the HC and patients with a history of HBV infection and HBsAg/anti-HBs seroconversion. Next, we assessed expression of the membrane-bound CD163 molecule. The available studies provide evidence for increased CD163 expression in peripheral blood monocytes in HBV-related liver failure [30, 31]. However, we did not find any studies addressing that particular issue in the subsequent phases of chronic HBV infection. In general, monocytes expressing the CD163 receptor are considered to have anti-inflammatory potential. Ye et al. reported that CD163 expression was significantly upregulated in HBV-related acute-on-chronic liver failure and chronic hepatitis B in comparison to the healthy population [31]. This observation in chronic hepatitis B is in line with our results, as we detected increased CD163 expression in classical and intermediate monocytes of CHB patients compared to the HC. Other research, that of Zhang et al. in HBV-related liver failure, demonstrated that expression of CD163 increases upon exposure to liver activated myofibroblasts in a PGE2-dependent manner. The authors proposed that the increase in CD163 expression in liver failure might be a protective mechanism counteracting inflammation and shifting monocytes towards the anti-inflammatory state [30]. Interestingly, in the CHB group, the expression of CD163 on monocytes increased in parallel with improved immune control of the HBV infection in HBsAg-positive patients and individuals with a history of HBsAg/anti-HBs seroconversion. As we can learn from previous studies

Table 4: Correlation of VL, qHBsAg, ALT, and APRI with CD163 expression on monocytes, sCD163, and sTWEAK.

Correlations	HBV-DNA VL (IU/mL)	qHBsAg (IU/mL)	ALT (U/L)	APRI
	r	*r*	*r*	*r*
	p	*p*	*p*	*p*
MFI of CD163+ on CD14++CD16- cells	-0.097	-0.052	**-0.310**	**-0.594**
	0.504	0.724	**0.015**	**0.015**
MFI of CD163+ on CD14++CD16+ cells	-0.103	0.077	**-0.288**	-0.203
	0.477	0.603	**0.024**	0.451
MFI of CD163+ on CD14+CD16++ cells	0.027	-0.107	0.083	0.311
	0.854	0.471	0.526	0.260
MFI of CD163+ on CD14+ cells	-0.079	-0.055	**-0.277**	-0.481
	0.586	0.710	**0.031**	0.059
sCD163 (ng/mL)	-0.0535	-0.181	**0.231**	**0.609**
	0.703	0.173	**0.046**	**0.006**
sTWEAK (pg/mL)	0.133	0.099	-0.037	-0.254
	0.319	0.458	0.761	0.293

supported by our observations, membrane-bound CD163 inversely correlates with sCD163 [32]. In the inflammatory environment CD163 is shed from the cell surface and sCD163 increases correspondingly. Lately, serum sCD163 was proposed as a prognostic factor for overall survival in liver cirrhosis, as well as a marker for hepatic inflammation and necrosis [20, 33, 34]. Dultz et al. observed significantly increased serum sCD163 concentrations in patients with ENH compared to HBsAg carriers (ENI). Moreover, serum sCD163 correlated positively with serum HBV-DNA, ALT levels, hepatic necroinflammatory activity index and fibrosis score [33]. Corresponding results were noted in the study of Laursen et al. in a group of HBeAg-positive and HBeAg-negative CHB patients [20]. In our study, we found higher serum concentration of sCD163 in HBV-infected patients vs. the control group. However, the status of immune control of infection did not affect serum concentrations of sCD163 within HBsAg-positive groups. We omitted histopathological assessment of liver biopsies due to the inadequate number of liver samples, which we address as a limitation of our study. Despite that fact, we observed a trend towards higher plasma sCD163 concentration in patients with portal/-periportal necroinflammatory activity in liver tissue ($G \geq 2$) in comparison to discrete portal inflammation ($G = 1$). Nevertheless, we attempted to assess the link between sCD163 and indirect biomarkers of hepatic necroinflammatory activity. We observed significant correlations between serum sCD163 level and ALT or APRI score, universal serological noninvasive markers of liver inflammation, and fibrosis. Considering the lack of correlations between the expression of CD163 on monocytes or serum sCD163 level and viral-associated parameters such as VL and qHBsAg, we presume that the alterations within CD163 expression and sCD163 level might result from the systemic inflammatory response rather than direct interaction of the virus with the population of monocytes. This hypothesis might be supported by the observation of Laursen et al. in CHB patients, which showed a decrease of sCD163 levels along with diminished ALT activity after antiviral therapy in both treatment responders and nonresponders [20].

We noted a lower plasma sTWEAK concentration in chronic HBV infection in comparison to the healthy controls. Despite lower plasma sTWEAK levels in the CHB group, these patients exhibited a trend towards higher plasma sTWEAK in individuals with portal/periportal necroinflammatory activity in liver tissue ($G \geq 2$) vs. discrete portal inflammation ($G = 1$). Our findings correspond to the results of Asil and Dertli, which also showed a reduced concentration of sTWEAK in CHB and its gradual increase along with the degree of inflammation and fibrosis in the liver biopsies of HBV-infected patients. The authors provide a consistent hypothesis that the upregulation of Fn14 receptors in inflammatory conditions leads to the uptake of circulation sTWEAK, thus diminishing its serum concentration in chronic hepatitis B [35]. Based on our findings, we presume that the upregulation of the CD163 molecule on monocytes might also contribute to the serum sTWEAK decrease in CHB. Apart from binding to the Fn14 molecule, TWEAK may interact with the membrane receptor CD163 as an antagonist ligand, thus resulting in inhibition of monocyte activation. On the other hand, there is evidence that the CD163 molecule present on monocytes can serve as a scavenger receptor for TWEAK, thus blocking biological functions of the TWEAK-Fn14 axis, e.g., apoptotic cell death [23]. Here, we observed negative correlations between expression of the CD163 receptor on all studied populations of monocytes and sTWEAK plasma concentration. That observation could justify the laboratory evidence of putative mutual regulatory interactions of both molecules, as increased membrane CD163 expression probably contributes to the decline of serum concentration of sTWEAK. This hypothesis may shed some light on the observation of a decreased sTWEAK level parallel to increased monocyte CD163 expression in CHB but does not explain the paradoxical gradual increase of sTWEAK along with the progression of liver inflammation. As we mentioned before, in inflammatory conditions,

CD163 is shed from the cellular membrane, thus increasing the sCD163 level and the sTWEAK level, as the expression of membrane CD163 binding sTWEAK molecules is diminished. With regard to liver fibrosis, we did not find any correlations between monocyte CD163 expression, sCD163, and sTWEAK. However, the latter observation is biased by the inadequate number of liver biopsy samples, which does not represent all stages of progression of liver fibrosis. Therefore, in line with other authors, we might presume that expression of CD163 on monocytes and sCD163 level reflect the current inflammatory status and activity of fibrogenesis rather than advancement of disease in general [36].

In conclusion, our results show that monocytes are an essential component of the immune reaction in chronic hepatitis B. As CD163 is considered as a marker of monocytes with anti-inflammatory properties counteracting chronic inflammation, in CHB, significantly higher frequencies of classical and intermediate monocytes' CD163 expression were observed. The expression of CD163 on classical monocytes increased in parallel with improved immune control of the HBV infection. Patients who seroconverted HBsAg had the highest expression of CD163, which suggests involvement of monocytes in immune control of HBV infection. On the other hand, our data suggest that persistent inflammation is accompanied by lower CD163 expression, which may be associated with impairment in activation of monocytes. Moreover, the decline of "protective monocytes" expressing CD163 molecule results in impaired neutralisation of TWEAK, thus allowing proinflammatory and profibrogenic TWEAK-Fn14 interactions. This appears of great interest with respect to improvement of the management of liver inflammation in general. Further studies are required to reveal whether the targeting of the CD163-TWEAK axis might be a potential strategy for treating liver inflammatory diseases.

5. Conclusions

In CHB, significantly higher frequencies of classical and intermediate monocytes' CD163 expression were observed. The expression of CD163 on classical monocytes increased in parallel with improved immune control of the HBV infection. Patients who seroconverted HBsAg had the highest expression of CD163 on monocytes, which suggests involvement of monocytes in immune control of HBV infection. Persistent inflammation is accompanied by higher CD163 expression and sCD163 level and lower sTWEAK level, which may be associated with improper activation of monocytes and may contribute to the progression of liver disease.

Abbreviations

HBV: Hepatitis B virus
TWEAK: Tumour necrosis factor-like weak inducer of apoptosis
sCD163: Soluble CD163
sTWEAK: Soluble tumour necrosis factor-like weak inducer of apoptosis
CHB: Chronic HBV infection
RES: Spontaneously resolved HBV infection with HBsAg/anti-HBs seroconversion
S-CONV: Treatment-associated seroconversion HBsAg/anti-HBs
ENI: HBeAg-negative chronic infection
ENH: HBeAg-negative chronic hepatitis B
SUPR: ENH during nucleos(t)ide analogue therapy with complete HBV-DNA suppression
ENH-LR: Low-replicative hepatitis
ENI-HR: High-replicative patients with normal aminotransferases
HC: Healthy control group
HBV-VL: HBV viral load
qHBsAg: Quantitative HBsAg
ALT: Alanine aminotransferase
APRI: AST to platelet ratio index
ELISA: Enzyme-linked immunosorbent assays.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Disclosure

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Conflicts of Interest

The authors declare that there is no conflict of interests regarding the publication of this paper.

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