Soluble CD163 and CD163 Expression on Monocytes Associated with Chronic Hepatitis B Inflammation and HBsAg Loss

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Abstract

Background and Aims: Monocyte/macrophage-associated CD163 is an indicator of the severity of liver inflammation and cirrhosis, but the difference of soluble CD163 (sCD163) levels in chronic hepatitis B (CHB) patients and hepatitis B surface antigen (HBsAg)-loss patients is unclear. Herein, we aimed to compare the sCD163 levels in CHB patients and HBsAg-loss patients with and without antiviral treatment. Methods: sCD163 and CD163 expression on monocytes were compared among four groups, healthy subjects, treatment-naïve CHB patients, spontaneous HBsAg-loss patients, and treatment-related HBsAg-loss patients. The correlation between sCD163 levels and clinical parameters in CHB patients was analyzed. A group of 80 patients with hepatitis B virus (HBV) infection and liver biopsy were recruited. Results: sCD163 levels were higher in the CHB group than in the other three groups. sCD163 levels were higher in treatment-related HBsAg-loss patients than in spontaneous HBsAg-loss patients. sCD163 levels were negatively correlated with hepatitis B e-antigen (HBeAg) and HBsAg levels in HBeAg-positive patients. Liver biopsy results further demonstrated that sCD163 levels were elevated in CHB patients with substantial inflammation (A≥2) or fibrosis (F≥2). The sCD163 model was more sensitive in predicting inflammation than other noninvasive models. Its levels were higher in patients with normal alanine aminotransferase levels and significant inflammation (A≥2) than in patients with no or mild inflammation. Conclusions: sCD163 and CD163 expression on monocytes were associated with CHB inflammation and HBsAg loss, and may be used as markers to predict HBV-specific immune activation.

Citation of this article: Xie P, Yao B, Huang D, Chen Y, Gong Q, Zhang X. Soluble CD163 and CD163 Expression on Monocytes Associated with Chronic Hepatitis B Inflammation and HBsAg Loss. J Clin Transl Hepatol 2022;10(6):1059–1067. doi: 10.14218/JCTH.2021.00496.

Introduction

Hepatitis B virus (HBV) infection is a major health concern worldwide. Twenty million patients have been estimated to suffer from chronic HBV infection in China and are at a high risk of cirrhosis and hepatocellular carcinoma. Treatment with nucleos(t)ide analogues and pegylated interferon are effective in preventing HBV replication, but virus eradication has not yet been achieved.¹

Innate and adaptive immunity play important roles in HBV infection.² Antigen-presenting cells (APCs) bridge innate and adaptive immunity. Peripheral monocytes/macrophages and Kupffer cells in the liver are important APCs in the innate immune system that clear viral infection by activating HBV-specific CD4⁺ or CD8⁺ T cells.³,⁴ Monocytes/macrophages can be divided into several subsets according to the expression of cell surface antigens CD14 and CD16, including classical, intermediate, and nonclassical monocyte subsets.³ Monocytes/macrophages express the surface molecule CD163, which is a high-affinity scavenger receptor of the hemoglobin-haptoglobin complex,⁶ and in the absence of haptoglobin, with lower affinity, for hemoglobin alone.⁷ It is also a marker of cells of the monocyte/macrophage lineage.⁸ Activated monocytes/macrophages shed the hemoglobin-haptoglobin scavenger receptor CD163 into circulation as soluble CD163 (sCD163). Thus, sCD163 is a
marker of monocytes/macrophages activation and is negatively correlated with the expression of CD163 on monocytes/macrophages.9,10

Recently, it has been found that CD163 and sCD163 have diagnostic and predictive value in many infectious diseases.11–15 sCD163 has been reported as an indicator of liver inflammation and fibrosis in patients chronically infected with HBV.16 In addition, a study showed that sCD163 was independently associated with fibrosis in patients with chronic viral hepatitis B and C.11 Although CD163 and sCD163 are dependently associated with fibrosis in patients with chronic hepatitis B (CHB) or hepatitis B surface antigen (HBsAg) loss remain unclear. Comparison of CD163 levels between CHB and HBsAg-loss patients may be helpful in elucidating the dynamic change of CD163 in chronic HBV infection and provide a new marker for evaluating the specific immune status of patients with chronic HBV infection.

Methods

Selection of patients

A total of 170 adults were enrolled in the study between 2014 and 2016, including 24 healthy subjects (HS), 97 treatment-naïve CHB patients (CHB), 18 patients with spontaneous HBsAg loss (SL), and 31 patients with treatment-related HBsAg loss (TL). An additional 80 liver biopsy patients with chronic HBV infection were enrolled. Inclusion criteria for CHB patients were clinical, biochemical, and virological evidence of HBsAg positivity for at least 6 months, elevated alanine transaminase (ALT) and HBV DNA, not currently receiving antiviral therapy, or discontinuation of antiviral therapy more than 6 months previously and followed by a virological relapse. Virological response was defined as an undetectable HBV DNA level (<500 IU/mL) after 48 weeks of nucleos(t)ide analog therapy. Additional 80 liver biopsy patients with chronic HBV infection were enrolled. Inclusion criteria for CHB patients were clinical, biochemical, and virological evidence of HBsAg positivity for at least 6 months, elevated alanine transaminase (ALT) and HBV DNA, not currently receiving antiviral therapy, or discontinuation of antiviral therapy more than 6 months previously and followed by a virological relapse. Virological response was defined as an undetectable HBV DNA level (<500 IU/mL) after 48 weeks of nucleos(t)ide analog therapy. All subjects were recruited at Ruijin Hospital (Shanghai, China) and were negative for antibodies against hepatitis A, C, and delta viruses and human immunodeficiency virus. Patients with liver cirrhosis and/or systemic diseases were excluded from the study.

Biochemical and serologic assays

HBsAg, hepatitis B e-antigen (HBeAg), anti-HBs, anti-HBe, and hepatitis B core antibody (anti-HBc) were determined with a chemiluminescent microparticle immunoassay (Abbott Architect; Abbott Laboratories, North Chicago, IL, USA). Quantitative HBsAg (Abbott Diagnostics Division, Ireland) was assayed by the Abbott Architect system. Serum HBV DNA was quantified with Cobas AmpliPrep/Cobas TaqMan (Roche Diagnostics, Basel, Switzerland). Biochemical assays, such as ALT, aspartate transaminase (AST), platelet count (PLT), total bilirubin (TBIL), and direct bilirubin (DBIL) were also performed at the time of sampling.

Isolation of peripheral blood monocytes

Peripheral blood mononuclear cells (PBMCs) were isolated using Lymphoprep (Axis Shield, Dundee, Scotland) within 4 h after blood collection, cryopreserved in fetal bovine serum (Gibco, USA) containing 10% dimethylsulfoxide (Sigma-Aldrich, St. Louis, MO, USA), and stored in liquid nitrogen.

Determination of sCD163 and CD163 expression on monocytes

sCD163 was determined with an enzyme-linked immunosorbent assay (ELISA). sCD163 in plasma was measured with a Duoset ELISA kit (R&D Systems, Minneapolis, MN, USA) following the manufacturer’s instructions. CD163 expression on monocytes was assayed by flow cytometry. To correlate the expression of CD163 with that of other monocyte cell surface markers, PBMCs were assayed by flow cytometry after surface antibody staining of CD14 (anti-human, FITC, Mouse IgG2a, κ, Biolegend), CD16 (anti-human, PE, Mouse IgG1, κ, Biolegend), CD163 (anti-human CD163, APC, Mouse IgG1, κ, Biolegend), incubated at 4°C for 30 min, washed once with phosphate buffered saline, fixed with 100 μL 4% paraformaldehyde. A FACSCalibur flow cytometer (BD, San Diego, USA) was used with FlowJo 9.6 software (Tree Star). The mean fluorescence intensity (MFI) of CD163+ cells were detected.

Model calculations

The METAVIR score is used to evaluate the severity of inflammation and fibrosis. The grade indicates the amount of inflammation in the liver and the stage represents the amount of scarring or fibrosis. The gamma-glutamyl transpeptidase to platelet ratio (GPR), red cell distribution width-to-platelet ratio (RPR), fibrosis index based on four factors (FIB-4), and aspartate aminotransferase-to-platelet ratio (APRI) were calculated as: GPR = (GGT/ULN of GGT) × 100/PLT; RPR=RDW (%)/PLT; FIB-4=(age × AST) / (PLT × ALT1/2); and APRI = (AST/ULN of AST) ×100/PLT where ULN is the upper limit of normal. The AAG and AAGP diagnostic algorithm for the evaluation of significant liver inflammation were calculated as previously described.17

Statistical analysis

One-way analysis of variance with the Bonferroni correction was used for the multiple-group comparisons. Student’s t-test was used to compare between-group differences in normally distributed variables. Kruskal-Wallis and Mann-Whitney tests were used for non-normally distributed data. The correlation coefficient (r) was calculated with the non-parametric Spearman correlation. Statistical significance was set at p<0.05. Numerical data were reported as mean ± standard error of the mean.

Results

Demographic, serological, biochemical characteristics and sCD163 levels of the subjects

Baseline demographic, serological, and biochemical characteristics for the subjects are shown in Supplementary Table 1. The four groups were healthy subjects, treatment-naive patients with CHB, patients with spontaneous HBsAg loss, and patients with treatment-related HBsAg loss. CHB patients had the highest ALT, AST, TBIL, and DBIL levels (p<0.001) and the lowest PLT levels (p<0.001); 67% were genotype B and 23% were genotype C. The proportion of HBeAg-positive pa-
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...the plasma sCD163 levels in patients with treatment-related HBsAg loss (558.6±58.68 ng/mL) were significantly higher than those in patients with spontaneous HBsAg loss (255±29.21 ng/mL; p=0.003). Taken together, the results indicate that sCD163 may be a sensitive serum marker for CHB and may be used as an index for evaluating HBV infection prognosis.

**Correlation of plasma sCD163 level and clinical parameters of CHB patients**

The correlations between plasma sCD163 levels and clinical parameters of CHB patients are shown in Figure 2A–E. Plasma sCD163 levels were positively correlated with ALT (r=0.69, p<0.001), AST (r=0.77, p<0.001), DBIL (r=0.55, p<0.001), and TBIL (r=0.25, p=0.016) and negatively correlated with PLT (r=−0.31, p=0.004). The correlations indicate that sCD163 may be associated with liver inflammation. In HBeAg-positive patients, sCD163 levels were negatively correlated with HBeAg (r=−0.38, p=0.0019; Fig. 2F), HBsAg (r=−0.45, p<0.001; Fig. 2G), and HBV DNA (r=−0.08, p=0.52; Fig. 2H). In HBeAg-positive CHB patients, the HBsAg and HBV DNA levels decreased with the progression of immune activation. The negative correlation of sCD163 levels with HBsAg and HBV DNA reflects its...
Expression of membrane-bound CD163 on monocytes

We assayed the presence of CD163+ monocytes in the 101 subjects in the four study groups who had PBMCs sufficient for multiparameter flow cytometry. The monocyte population was divided into classical (CD14+CD16−; Fig. 3A), intermediate (CD14+CD16+; Fig. 3B), and nonclassical (CD14−CD16+; Fig. 3C) monocyte subsets. No statistically significant differences were observed in the percentages of classical (p=0.410), intermediate (p=0.617), and nonclassical (p=0.192) monocytes in the four groups (Table 1). The frequency of CD163 expression (Table 1) reveal no significant differences on classical (p=0.568), intermediate (p=0.555), and nonclassical (p=0.264) monocytes. However, the MFI of CD163 expression was significantly different on the classical (p=0.010) and intermediate (p=0.042) monocytes but not on nonclassical monocytes (p=0.098, Table 1). Interestingly, as shown in Figure 4D, in classical monocytes, the MFI of CD163 expression in healthy subjects was significantly higher than that in patients with CHB (232.2±29.61 vs. 138.9±7.4, p<0.01), spontaneous HBsAg loss (232.2±29.61 vs 146.9±19.45, p<0.01), and treatment-related HBsAg loss (232.2±29.61 vs. 142.1±11.34, p<0.01). In intermediate monocytes, the MFI of CD163 expression in healthy subjects was higher than that in patients with CHB (187.9±20.21 vs. 163.3±10.20, p<0.01) and in those with spontaneous HBsAg loss (187.9±20.21 vs. 121.7±13.12, p<0.01) but not in those with treatment-related HBsAg loss (187.9±20.21 vs. 168.9±21.37, p=0.625).

Correlation between plasma sCD163 levels and liver inflammation and fibrosis in chronic HBV-infected patients with liver biopsy

The clinical parameters, including baseline demographic, serological, and biochemical characteristics on the 80 liver biopsy patients with HBV infection are shown in Supplementary Table 2. The correlation between sCD163 levels and HBV was assessed with the meta-analysis of histological data in viral hepatitis (METAVIR) scores in each group. We compared the sCD163 levels in those with no or mild inflammation and fibrosis (A<2 and F<2) and those moderate-to-severe inflammation or fibrosis (A<2 or F≥2). As shown in Figure 3A, sCD163 levels were higher in moderate or severe inflammation or fibrosis (A<2 or F≥2) than in no or mild inflammation and fibrosis (A<2 and F<2; p<0.01). In addition, our results showed that sCD163 levels were significantly associated with moderate and severe inflammatory activity in liver biopsy (A<2 vs. A≥2; p=0.02; Fig. 3B). sCD163 levels were also significantly higher in moderate and severe liver fibrosis than no or mild fibrosis (F<2 vs. F≥2; p<0.01).
vs. F≥2; \( p<0.001 \); Fig. 3C). We also observed that despite significant liver inflammation (A≥2), several CHB patients had normal ALT levels, which indicates that ALT had a false-negative rate in predicting significant liver inflammation. As shown in Figure 3D, the levels of sCD163 were compared between patients with HBV infection (A<2) and patients with CHB (A≥2) and normal ALT levels. The results show that although ALT levels were normal, sCD163 levels were significantly higher (\( p=0.04 \)) in patients with moderate or severe inflammation (A≥2) than in those with no or mild inflammation (A<2). However, sCD163 levels were not significantly different in those no or mild inflammation (A<2) and moderate or severe inflammation (A≥2) under abnormal ALT levels (Fig. 3E). These results suggest that sCD163

| Table 1. Expression of membrane-bound CD163 on monocytes |
|-----------------------------------------------------------|
| Healthy subjects (N=9) | Treatment-naive CHB patients (N=53) | Patients with spontaneous HBsAg loss (N=13) | Patients with treatment-related HBsAg loss (N=26) | P-value |
| CD14^+CD16^- (%) | 3.2±0.92 | 4.3±0.43 | 3.9±1.09 | 4.8±0.72 | 0.410 |
| CD14^+CD16^-CD163^+ (%) | 61.5±7.93 | 57.2±2.68 | 62.3±4.45 | 62.3±3.12 | 0.568 |
| CD14^+CD16^-CD163^+MFI | 232.2±29.61 | 138.9±7.40 | 146.9±19.45 | 142.1±11.34 | 0.010 |
| CD14^-CD16^- (%) | 0.9±0.19 | 1.1±0.12 | 0.9±0.19 | 1.2±0.18 | 0.617 |
| CD14^-CD16^-CD163^- (%) | 71.8±4.34 | 64.2±2.70 | 63.6±3.16 | 66.4±3.55 | 0.555 |
| CD14^-CD16^-CD163^-MFI | 187.9±20.21 | 162.3±10.20 | 121.7±13.12 | 168.9±21.37 | 0.042 |
| CD14^-CD16^- (%) | 10.7±2.12 | 13.5±0.83 | 11.6±1.51 | 10.9±1.11 | 0.192 |
| CD14^-CD16^-CD163^- (%) | 2.5±0.9 | 1.2±0.23 | 0.9±0.29 | 1.4±0.51 | 0.264 |
| CD14^-CD16^-CD163^-MFI | 74.5±16.40 | 87.6±5.22 | 73.2±12.07 | 74.4±8.45 | 0.098 |

The monocyte expression is expressed as a percentage. Data are mean±standard error of the mean. HBsAg, hepatitis B surface antigen.

Fig. 4. Mean fluorescence intensity (MFI) of CD163 expression on monocytes subsets. Gating of monocyte/macrophage subsets by CD14 and CD16 expression. (A) CD14^+CD16^-; (B) CD14^+CD16^-; (C) CD14^-CD16^- and intermediate (CD14^-CD16^-CD163^-) monocyte subsets in healthy subjects, treatment-naive CHB patients, patients with spontaneous HBsAg loss, and patients with treatment-related HBsAg loss. Data are mean±SEM. \( p \)-values <0.05 are in italic. CHB, chronic hepatitis B; HBsAg, hepatitis B surface antigen; sCD163, soluble CD163.
levels were more accurate than ALT levels in predicting liver inflammation. Similarly, the levels of sCD163 were higher in patients with moderate or severe fibrosis (F≥2) than in those with no or mild fibrosis (F<2) under normal ALT levels (p=0.035; Fig. 3F) but were not significantly different between the two groups with abnormal ALT levels (Fig. 3G).

**Predictive value of sCD163 levels in chronic HBV-infected patients with liver biopsy**

It is well known that when the liver has severe inflammation, most patients with CHB have abnormal changes in liver function, including elevated ALT and AST levels, and fluctuation of HBsAg and HBV DNA. Receiver operating characteristic (ROC) curve analysis was used to evaluate the sensitivity of sCD163, ALT, AST, HBsAg, and HBV DNA in detecting significant liver inflammation (Fig. 5A). The areas under the ROC curve (AUROCs) of sCD163, ALT, AST, HBsAg, and HBV DNA for differentiating patients with A≥2 from those with A<2 were 0.68 (p=0.008), 0.68 (p=0.008), 0.68 (p=0.008), 0.55 (p=0.48), and 0.62 (p=0.06), respectively. Multiple logistic regression analysis was performed with the METAVIR inflammation score as the dependent variable and sCD163, ALT, and AST as the explanatory variables. The ROC curves are shown in Figure 5B, and the new AUROC was 0.70 (p=0.001). The results suggest that sCD163 combined with ALT and AST was a better predictor of liver inflammation. We further evaluated the performance of other noninvasive models for predicting significant liver inflammation. The AUROCs and 95% confidence intervals (CI) of the various noninvasive models are shown in Table 2. In general, the diagnostic performance of other noninvasive models was lower than that of our model. The results show that sCD163 was a better predictor of liver inflammation. In addition, the presence of significant fibrosis (F≥2) is normally used as an indicator for initiating antiviral therapy. We compared the AUROC of sCD163 with those of GPR, RPR, APRI, and FIB-4, as shown in Figure 5C. The AUROC

### Table 2. Comparison of AUROC between sCD163 model and other non-invasive models for liver inflammation

| Non-invasive models | AUROC  | 95%CI  |
|---------------------|--------|--------|
| sCD163 model        | 0.70   | 0.58–0.81 |
| AAGP model          | 0.61   | 0.48–0.74 |
| AAG model           | 0.6    | 0.47–0.73 |
| RPR model           | 0.58   | 0.43–0.74 |

sCD163, soluble CD163; RPR, red cell distribution width to platelet ratio; ROC, Receiver operating characteristic

![Fig. 5. Predictive value of sCD163 level in patients with chronic HBV infection and liver biopsy.](image-url)
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of sCD163 was 0.71 (95% CI: 0.597–0.824, \( p = 0.0012 \)), those of GPR, RPR, APRI, and FIB-4 were 0.70, 0.59, 0.68, and 0.54, respectively. The levels of sCD163 were significantly higher than those of GPR, RPR, APRI, and FIB-4 in the prediction of significant fibrosis. We performed multiple logistic regression analysis with the METAVIR fibrosis score as the dependent variable and sCD163, ALT, and AST as the explanatory variables. The ROC curves are shown in Figure 5D, and the new AUROC was 0.71 (\( p = 0.001 \)). The results suggest that sCD163 is a better predictor of liver fibrosis than GPR, RPR, APRI, or FIB-4.

**Prediction of antiviral treatment response using sCD163**

Some studies have reported that antiviral treatment significantly reduced the levels of sCD163.\(^{18}\) Our study further investigated whether sCD163 levels could predict antiviral treatment response. As shown in Figure 6A, defining virological response with the undetectable HBV DNA levels after 48 weeks of nucleos(t)ide analog treatment, there was no difference in the baseline levels of sCD163 at baseline between virological response (n=25) and nonresponse (n=8). Similarly, in Figure 6B, defining serological response a decrease of \( \geq 1 \log_{10} \) IU/mL in HBSAg after 48 weeks of nucleos(t)ide analog treatment, there were no difference in the baseline levels of sCD163 in the serological responders (n=11) and nonresponders (n=14; \( p < 0.01 \)), which indicates that the correlation between sCD163 levels and antiviral response needs further investigation.

**Discussion**

Our study demonstrated that monocyte/macrophage expression and sCD163 levels were closely correlated with liver inflammation and fibrosis in HBV infection. Importantly, we revealed a correlation between CD163 expression and immune activation in patients with CHB. The ultimate endpoint of chronic HBV treatment was sustained HBsAg loss with or without seroconversion to hepatitis B surface antibody (anti-HBs). Current antiviral therapies using pegylated interferon or nucleos(t)ide analogues to suppress HBV replication and improve the prognosis of CHB, but they fail to clear HBsAg. As HBsAg might contribute to the impairment of innate and adaptive immunity and the exhaustion of T cell and B cell responses, a reduction of serum HBsAg could facilitate the recovery of the host’s immune system.\(^{19}\) Our results showed that the levels of sCD163 were higher in patients with CHB than in HBsAg-loss patients and healthy subjects. Interestingly, we found that sCD163 levels were lower in patients with spontaneous HBsAg loss than in those...
with treatment-related HBsAg loss. However, the mechanism underlying the lower levels of sCD163 in patients with spontaneous HBsAg loss needs to be further explored.

In patients with CHB, sCD163 was negatively correlated with HBsAg and HBeAg in HBeAg-positive patients, which reflects its correlation with immune activation considering that HBsAg and HBeAg levels decreased with the progression of immune activation in HBeAg-positive patients. Similarly, in HBeAg-negative patients with CHB, the levels of HBV DNA fluctuated periodically or increased with the progression of immune activation, and the positive correlation between sCD163 and HBV DNA reflected the correlation between sCD163 and immune activation during HBV infection. The results indicate that sCD163 is closely associated with immune activation during HBV infection.

Some studies have reported that sCD163 is an indicator of liver inflammation and fibrosis in patients with HBV infection.\textsuperscript{11–13,16,20} Our study further demonstrated that sCD163 levels were positively correlated with the severity of liver inflammation and fibrosis in patients with CHB. Importantly, we found that sCD163 was higher in CHB patients with significant inflammation (A≥2) than in those with no or mild inflammation (A<2) with normal ALT levels. The results suggest that sCD163 was more accurate than ALT in identifying significant liver inflammation. Meanwhile, ROC analysis showed that sCD163 combined with ALT and AST was a better predictor of liver inflammation. In addition, we compared the AUC of sCD163 with those of GPR, RPR, APRI, and FIB-4, and found that sCD163 had the highest AUROC value, suggesting that sCD163 was a better predictor of liver fibrosis than ALT.

When inflammation and fibrosis were more severe, the levels of sCD163 were elevated, suggesting the activation of monocytes. Some studies have demonstrated that patients with CHB and no, mild, or severe hepatitis tended to have increased T cell activation linked with liver inflammation.\textsuperscript{21} Therefore, the results indicate that as a marker of monocyte activation, CD163 is associated with the activation of HBV-specific T cells.

Because of the strong interplay between specific T cell immunity and elimination of HBV, restoration of T cell immunity against HBV is considered an important outcome in current novel therapeutic approaches. Bertolletti et al. reported that the ability of HBV-specific CD8\textsuperscript{+} T cells to secrete Type 1 T helper cytokines in patients with spontaneous HBsAg loss was significantly higher than that in patients with chronic HBV infection.\textsuperscript{22,23} HBV-specific immunity in patients with resolved HBV infection is robust and multifunctional, whereas CHB is characterized by dysfunctional innate and adaptive antiviral immunity.\textsuperscript{24} Although regular antiviral therapy can partially restore the host-specific immune response, it cannot completely reconstruct the host’s HBV-specific immune function.\textsuperscript{25} In addition, more effective immunotherapy and representative markers are needed to reflect immune status in patients with HBV infection. In our study, the decrease in sCD163 levels in patients with spontaneous HBsAg loss compared with patients with CHB may partly reflect the restoration of the host anti-HBV-specific immune response.

In addition, classical monocytes can phagocytize foreign pathogens and present antigens, intermediate monocytes mainly have pro-inflammatory functions, and nonclassical monocytes produce anti-inflammatory cytokines.\textsuperscript{26–28} T cells and classical monocytes are closely related, as the latter interacts with the former to induce T cell activation in target organs. Activated monocytes phagocytize other cells and are able to digest their proteins and present them to T cells, which recognize the molecular signatures of particular proteins and activate an immune response.\textsuperscript{29} CD163, a specific monocyte marker, is closely associated with the activation of the immune response. In patients with HBV infection, antigen-presentation by classical monocytes and inflammatory factor secretion by intermediate monocytes are involved in the establishment of HBV-specific immunity. Our study showed that the MFI of CD163 expression in classical and intermediate monocytes was higher in healthy subjects than in patients with HBV infection. The results indicate monocyte activation with HBV infection and that activated monocytes shed the hemoglobin-haptoglobin scavenger receptor CD163 into circulation as sCD163. Therefore, the expression of CD163 on monocytes may be a potential marker of HBV-specific immune activation.

As previously described, monocytes are essential for the establishment of innate and adaptive immune responses. Progression status in HBV infection is strongly associated with monocyte activation, but it is necessary to elucidate how the virus directly or indirectly modifies monocyte function and how the altered function affects the HBV-specific immune response. Importantly, we need to further understand the mechanism of the immune response and the dynamic changes in CD163 expression during immunotherapy. Based on the results of our study, CD163 may be used as a marker, which is of great significance for assessing immune activation in patients with HBV infection.

In summary, this study indicated that expression of sCD163 and CD163 expression on monocytes was associated with CHB inflammation and HBsAg loss, and might be used as more sensitive markers to predict HBV-specific immune activation and worthy of further validation in larger groups of patients. The study included only CHB genotype B and genotype C patients, and it was descriptive. The underlining mechanisms need to be further investigated.

Acknowledgments

We would like to thank Dr Ren Zhu and Professor Geneviève Inchauspé for their helpful discussion in the study.

Funding

This work was supported by funding from the Transgene SA, International Cooperation, a grant from the Shanghai Scientific and Technology Committee (16410711900), and National Nature Science grants (81672069 and 81974301).

Conflict of interest

XZ has been an editorial board member of Journal of Clinical and Translational Hepatology since 2013. The other authors have no conflict of interests related to this publication.

Author contributions

Conception and design of the study (XZ, QG), collection of the data (DH, BY, YC, QG), analysis of the data (PX, DH), and writing of the paper (PX, XZ).

Ethical Statement

The study was approved by the Ethics Committee of Ruijin Hospital and was conducted following the ethical guidelines of the Declaration of Helsinki.

Data sharing statement

All data are available upon request.
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References

[1] Tsounis EP, Tourkochristou E, Mouzaki A, Triantos C. Toward a new era of hepatitis B virus therapeutics: The pursuit of a functional cure. World J Gastroenterol 2021;27(21):2727–2757. doi:10.3748/wjg.v27.i21.2727, PMID:34139551.

[2] Maini MK, Gehring AJ. The role of innate immunity in the immunopathology of treatment of HBV infection. J Hepatol 2016;64(1 Suppl):S60–S70. doi:10.1016/j.jhep.2016.01.028, PMID:27084038.

[3] Auffray C, Sieweke MH, Geissmann F. Blood monocytes: development, heterogeneity, and relationship with dendritic cells. Annu Rev Immunol 2009;27:669–692. doi:10.1146/annurev.immunol.021908.132557, PMID:19132917.

[4] Boltjes A, Movita D, Boonstra A, Woltman AM. The role of Kupffer cells in hepatic? and hepatitis C virus infections. J Hepatol 2014;61(3):660–671. doi:10.1016/j.jhep.2014.04.026, PMID:24798624.

[5] Krijgsman D, De Vries NL, Andersen MN, Skovbo A, Tollenaar R, Moller HJ, et al. CD163 484 as a Biomarker in Colorectal Cancer: The Expression on Circulating Monocytes and Tumor-Associated Macrophages, and the Soluble Form in the Blood. Int J Mol Sci 2020;21(16):5929. doi:10.3390/ijms21165929, PMID:32824692.

[6] Kristiansen M, Graversen JH, Jacobsen C, Sonne O, Hoffman HJ, Law SK, et al. Identification of the haemoglobin scavenger receptor. Nature 2001;409(6817):198–201. doi:10.1038/35051594, PMID:11966444.

[7] Scher D, Scher CA, Buehler PW, Boykins RA, Schoedon G, Aylash AI, et al. CD163 is the macrophage scavenger receptor for native and chemically modified hemoglobin in the absence of haptoglobin. Blood 2006;107(1):373–380. doi:10.1182/blood-2005-03-1014, PMID:16189277.

[8] Lau SK, Chu PG, Weiss LM. CD163: a specific marker of macrophages in paraffin-embedded tissue samples. Am J Clin Pathol 2004;122(5):794–801. doi:10.1309/QHDE-YFBN-1KQX-UUHE, PMID:15491976.

[9] Semnani-Azad Z, Blanco Mejia S, Connelly PW, Bazinet RP, Retnakaran R, Jenkins DJA, et al. Soluble CD163 and mannose receptor associate with chronic hepatitis B activity and fibrosis and decline with treatment. J Gastroenterol Hepatol 2018;33(2):484–491. doi:10.1111/jgh.13849, PMID:28618015.

[10] Tout J, Laureiro D, Mansouri A, Sourettes V, Boyer N, Asselah T. Hepatitis B surface antigen seroclearance: Immune mechanisms, clinical impact, importance for drug development. J Hepatol 2020;72(3):409–422. doi:10.1016/j.jhep.2020.04.013, PMID:32333923.

[11] Davis BH, Zarev PV, Human monocyte CD163 expression inversely correlates with soluble CD163. Cytotherapy 2005;7(3):162–22. doi:10.1002/cytob.20031, PMID:16524200.

[12] Kazanov K, Carrera F, Filler M, Bibby BM, Vilstrup H, George J, et al. Soluble CD163, a macrophage activation marker, is independently associated with fibrosis in patients with chronic viral hepatitis B and C. Hepatology 2014;60(2):521–530. doi:10.1002/hep.27129, PMID:24623757.

[13] Ye H, Wang L, Zhao J, Wang K. Increased CD163 expression is associated with acute-on-chronic hepatitis B liver failure. World J Gastroenterol 2011;17(18):2818–2825. doi:10.3748/wjg.v17.i18.2818, PMID:23687420.

[14] Gronbaek H, Rodgaard-Hansen S, Aagaard NK, Arroyo V, Moestrup SK, Garcia E, et al. Macrophage activation markers predict mortality in patients with liver cirrhosis without or with acute-on-chronic liver failure (ACLF). J Hepatol 2016;64(4):813–822. doi:10.1016/j.jhep.2015.11.021, PMID:26639396.

[15] French AL, Grennan D, Daubert E, Seaberg EC, Peters M, Augenbraun M, et al. Decreases in markers of monocyte/macrophage activation after hepatitis C eradication in S18 HIV/hepatitis C virus coinfected women. AIDS 2021;35(9):1433–1438. doi:10.1097/QAD.0000000000002869, PMID:33710024.

[16] Sherman KE, Meeds HL, Rouster SD, Abdel-Hameed EA, Hernandez J, Tama-rgo J, et al. Soluble CD163 Identifies Those at Risk for Increased Hepatic Inflammation & Fibrosis. Open Forum Infect Dis 2021;8(6):ofab203. doi:10.1093/ofid/ofab203, PMID:34104667.

[17] Dultz G, Gerber L, Farnik H, Berger A, Vermehren J, Plei T, et al. Soluble CD163 is an indicator of liver inflammation and fibrosis in patients chronically infected with the hepatitis B virus. J Viral Hepat 2015;22(4):427–432. doi:10.1111/jvh.12309.

[18] Li Q, Zhou Y, Huang C, Li W, Chen L. A novel diagnostic algorithm to predict significant liver inflammation in chronic hepatitis B virus infection patients with detectable HBV 530 DNA and persistently normal alanine transaminase. Sci Rep 2018;8(1):15449. doi:10.1038/s41598-018-33412-z, PMID:30337643.

[19] Laursen TL, Wong GL, Kazankov K, Sandahl T, Moller HJ, Hamilton-Dutoit S, et al. Soluble CD163 and mannose receptor associate with chronic hepatitis B activity and fibrosis and decline with treatment. J Gastroenterology Hepatol 2018;33(2):484–491. doi:10.1111/jgh.13849, PMID:28618015.

[20] Tout J, Laureiro D, Mansouri A, Sourettes V, Boyer N, Asselah T. Hepatitis B surface antigen seroclearance: Immune mechanisms, clinical impact, importance for drug development. J Hepatol 2020;72(3):409–422. doi:10.1016/j.jhep.2020.04.013, PMID:32333923.