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Myeloid-Derived Suppressor Cells Regulate Immune Response in Patients with Chronic Hepatitis B Virus Infection through PD-1–Induced IL-10

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Although myeloid-derived suppressor cells (MDSCs) are well known for their immunosuppressive function in several pathological conditions, the role of MDSCs in hepatitis B virus infection remains obscure. In this study, we investigated the frequency and function of MDSCs in the peripheral blood and liver of 91 chronic hepatitis B (CHB) patients. A higher percentage of MDSCs, defined as CD14+ HLA-DRlow, was detected in peripheral blood of CHB patients than that of the healthy controls. Moreover, high expression of programmed death 1 (PD-1) and secretion of IL-10 in this population were determined. The frequency of MDSCs was positively correlated with serum viral load, but it was negatively correlated with liver inflammatory injury. These cells were also abundant in liver tissue of CHB patients and were related to necroinflammatory activity. Furthermore, we found that these cells could suppress hepatitis B virus–specific CD8+ T cell response, including reduced proliferation and IFN-γ production, and inhibit degranulation of CD8+ T cells, including reduced production of granzyme B and perforin. Importantly, PD-1–induced IL-10 production by MDSCs was responsible for the suppressive activity. To our knowledge, for the first time our study proved that CD14+HLA-DRlowPD-1+ MDSCs in CHB patients contribute to an inadequate immune response against the virus and lead to chronic infection, which represents a potential target for therapeutic intervention. The Journal of Immunology, 2014, 193: 5461–5469.

Hepatitis B virus (HBV) infection is a major global health problem, especially in Asia. There are >350 million people chronically infected with this virus worldwide, and in China ~22 million people suffer from chronic hepatitis B (CHB) virus (1). Accumulating evidence suggests that immune dysregulation occurs in CHB patients, and that persistent HBV infection could lead to increased risk of developing liver cirrhosis, liver failure, and liver cancer (2). HBV-specific CD8+ CTLs are essential for the control of HBV infection, but suppression of virus-specific T cells, demonstrated as functional impairment of proliferation and exhausted cytokine production, has been observed in patients with persistent HBV infection (3, 4). Thus, a better understanding of the mechanisms behind T cell suppression is critical for identifying effective strategies for HBV immunotherapy.

Recently, the focus of this area of research has shifted to myeloid-derived suppressor cells (MDSCs) owing to their prominent role in immunoregulation. MDSCs are a heterogeneous population of cells composed of precursors of macrophages, granulocytes, dendritic cells, and myeloid cells at earlier stages of differentiation (5). These cells have been demonstrated to have the potential to suppress immune responses of T cells by way of TGF-β, Arg1, programmed death ligand (PD-L) 1, and reactive oxygen species (ROS), as well as by regulatory T cell induction (6–11). In mice, MDSCs are characterized by the coexpression of Gr-1 and CD11b. Human MDSCs, although mostly described as Lin− HLA-DR−CD33+, have not been well characterized owing to lack of specific markers (12). Recently, a novel subset of monocytes defined as CD14+HLA-DR−lowPD-1+ MDSCs were closely associated with disease progression in malignancy and some inflammation-mediated pathological conditions (7, 16). Although recent studies report MDSC expansion in viral infections (17–22), phenotypic and functional features of MDSCs are still poorly defined in patients with CHB.

The aim of this study was to determine whether MDSCs are involved in the impaired immune response leading to chronic HBV infection. We hypothesized that patients with chronic HBV infection have a higher proportion of MDSCs compared with healthy controls and that MDSCs are responsible for the inability of infection clearance for patients. For this purpose, we investigated the frequency, phenotype, and function of MDSCs in chronic HBV patients and evaluated the association of MDSCs with disease conditions.
activity and viral clearance. These findings may provide new insights for understanding the impaired immune response in humans with virus infection.

Materials and Methods

Patients and healthy controls

Blood samples were obtained from 91 HBV-infected hepatitis B e Ag (HBeAg)* subjects, including 70 immune-activated (IA) patients and 21 immune-tolerant (IT) carriers. All patients were diagnosed according to previously described criteria (23). Briefly, the IT group was defined as patients with minimal liver damage, normal alanine aminotransferase (ALT) levels (<40 U/l), active viral replication (>10^4 IU/ml), and no inflammation in the liver. The IA group included patients with high levels of inflammation in the liver, elevated serum ALT levels (>40 U/l), but decreased viral replication (>100 IU/ml). None of the subjects with HBV infection included in the study had received antiviral therapy or immunosuppressive drugs within 6 mo before sampling. Liver biopsies were collected from 21 IA patients, and the degree of hepatic inflammation was graded by the modified histological activity index (HAI) (24). Briefly, two components, grading and staging, were given in a numerical value ranging from 0 to 4: grading was used to describe the intensity of necroinflammatory activity, and staging was a measure of fibrosis and architectural alteration in chronic hepatitis. Increased numerical values indicated a greater severity of disease. Individuals with coinfections of hepatitis C virus (HCV), hepatitis D virus, and HIV infections were excluded from enrollment. The study protocol was in accordance with the ethical guidelines of the 1975 Declaration of Helsinki and was approved by the Beijing 302 Hospital Research Ethics Committee. Written informed consent was obtained from all participants. Baseline clinical data are shown in Table I. For comparison, 37 uninfected healthy controls were age and sex matched to the enrolled patients.

Cell isolation and sorting

PBMCs were isolated from heparinized blood by Ficoll-Hyphaque (Pharmacia, Uppsala, Sweden) density gradient centrifugation. Monocyte counts of the patients were determined by automated hematology analysis (XK-21N, Sysmex). PBMCs were stained with Abs to human CD14 and HLA-DR. In selected assays, PBMCs were sorted into CD14^HLA-DR^low–depleted PBMCs, CD14^HLA-DR^-cells, or CD14^HLA-DR^-cells by using a MoFlo XDP cell sorter (Beckman Coulter). The purity of the monocytes after sorting was >97%.

Liver biopsy specimens were homogenized for isolation of intrahepatic leukocytes according to previously reported protocols (25), and then isolated leukocytes were washed with PBS and stained with the Abs.

Flow cytometric analysis

For surface marker staining, fresh heparinized peripheral blood (100 μl) was labeled with the following mAbs: PE-Cy7–conjugated PE-Cy5–conjugated HLA-DR, FITC-conjugated CD11b, FITC-conjugated CD11c, PE-conjugated CD33, PE-conjugated CD80, FITC-conjugated CD86, PE-conjugated PD-L1, PE-conjugated programmed death 1 (PD-1), PE-conjugated CD11b, and FITC-conjugated Lin1 (CD3, 14, 16, 19, 20, 56) (BioLegend, San Diego, CA). After the cells were incubated for 30 min at 4°C in the dark, they were lyzed with RBC lysis solution (BioLegend). Data acquisition and analysis were performed using a flow cytometer (FC500 MPL, Beckman Coulter) and FlowJo software (Tree Star, Ashland, OR). Isotype-matched Abs were used with all samples as controls.

For intracellular staining, these cells were incubated in RPMI 1640 medium supplemented with 10% FCS for 6 h as previously described (26). Monensin (0.4 μm, BD Pharmingen) was added during the first hour of incubation. After incubation, the cells were permeabilized with a Cytofix/Cytoperm kit (BD Biosciences) and stained with the corresponding intracellular Ab (27). Data acquisition and analysis were performed using a flow cytometer (FC500 MPL, Beckman Coulter) and FlowJo software (Tree Star). Isotype-matched Abs were used with all of the samples as controls.

Cytokine bead array

Cell culture supernatants were tested for levels of various cytokines as indicated. This was done using cytokine bead array (Bender MedSystems, Copenhagen, Denmark) according to the manufacturer’s protocol.

Detection of granzyme B and perforin production

For intracellular granzyme B (GrB) and perforin detection, cells cocultured were stimulated with PMA (50 ng/ml, Sigma-Aldrich), ionomycin (1 μg/ml, Sigma-Aldrich), and monensin (1 μg/ml, BD Biosciences) for 5 h as described previously (10). Cells were then stained with PE-Cy5–conjugated CD8^low cells, further permeabilized with a Cytofix/Cytoperm kit (BD Biosciences), and labeled with intracellular Ab FITC-conjugated perforin and PE-conjugated GrB (BD Biosciences).

Analysis of degranulation of CD8^T T cells

For determination of degranulation of CD8^T T cells, a CD107a mobilization assay was performed as described previously (28). After that, cells were washed, stained with PE-Cy7–conjugated CD8, and subjected to flow cytometric analysis.

Proliferation and IFN-γ secretion assays

For intracellular cytokine IFN-γ detection, cells cocultured were stimulated with PMA (50 ng/ml, Sigma-Aldrich), ionomycin (1 μg/ml, Sigma-Aldrich), and monensin (1 μg/ml, BD Biosciences) for 5 h as described previously (10). Cells were then stained with PE-Cy5–conjugated CD8, further permeabilized with a Cytofix/Cytoperm kit (BD Biosciences), and labeled with intracellular Ab FITC-conjugated IFN-γ (BD Biosciences).

Prior to coculture of PBMCs with CD14^HLA-DR^-low cells, PBMCs were labeled with CFSE according to the manufacturer’s instructions (Invitrogen). CFSE in the form of a 5 mM stock solution in DMSO was added to give a final concentration of 5 μM in the cell suspension. PBMCs cocultured with CD14^HLA-DR^-low cells at different ratios were then stimulated with anti-human CD3 and CD28 (both 1 μg/ml) or HBV core Ag (5 μg/ml) according to previous description (7). Cells were then washed, stained with PE-Cy7–conjugated CD8 at day 5, and subjected to flow cytometric analysis.

To further investigate the potential suppressive mechanisms by MDSCs, 10 μg/ml neutralizing anti-human PD-L1 Ab (BioLegend) or 10 μg/ml neutralizing anti-human IL-10 Ab (BioLegend) was added in the culture system. PBMCs and MDSCs were cocultured in Transwell plates (Corning) in indicated experiments.

Quantitative PCR

Total RNA from CD14^HLA-DR^-low cells was extracted using the RNeasy Mini kit (Qiagen, Hilden, Germany), and genomic DNA was removed using the RNase-free DNase set (Promega, Madison, WI). Quantitative PCR (SYBR Green Supermix, iCycler, Bio-Rad) was performed according to previously described protocols (29). Primer sequences for gene—specific amplifications were previously reported (7, 29). Relative gene expression was determined by normalizing to β-actin in each sample.

Serum HBV DNA, HBsAg, and HBeAg assays

The serum HBV DNA, HBsAg, and HBeAg assays were performed according to previous descriptions (25, 30, 31). The cut-off value was 100 IU/ml.

Statistical analysis

All data were analyzed using SPSS version 16.0 for Windows software (SPSS, Chicago, IL). Multiple comparisons were made between different groups with the Kruskal–Wallis H nonparametric test. Comparisons between various individuals were performed using the Mann–Whitney U test. Comparison between values within the same individual was performed using the Wilcoxon matched pairs r test. Correlation analysis was performed using Spearman rank correlation tests. For all tests, two-sided p < 0.05 was considered to be significant.

Results

Frequency and phenotype of MDSCs in CHB patients

We first compared the percentage of CD14^ monocytes present in the peripheral blood of chronic HBV patients (n = 91) with the percentage of those in healthy controls (n = 37) (Table I). As shown in Fig. 1A, there was a mild increase in the frequency of CD14^ monocytes in peripheral blood from patients with CHB, compared with that of healthy controls (Fig. 1A). Furthermore, when the CD14^ cells were analyzed for HLA-DR expression, we noticed a significant increase in the percentage of HLA-DR^-low cells, a novel population of MDSCs, in CHB patients as compared with healthy controls (Fig. 1B, 1C). Moreover, both IA and IT patients have an increased percentage of CD14^HLA-DR^-low cells in monocytes compared with healthy controls, whereas there...
was no significant difference between the two groups (Fig.1D).
The Lin^− HLA-DR^− CD33^+ cells in CHB patients, a phenotype
most commonly described for human cancer-associated MDSCs,
was found at low percentage and at similar levels as in healthy
controls (Supplemental Fig. 1).

The distribution of this subpopulation in liver tissue was further
determined in 21 patients with chronic infection. The frequency of
these cells was slightly higher in liver than that in the periphery
(8.97 ± 3.37% versus 7.52 ± 3.95%), albeit there is no statistical
significant difference, indicating that these cells were also abun-
dant in liver tissue (Fig. 1E).

Characterization of MDSCs in CHB patients
To further characterize the MDSCs in CHB patients, we compared
their phenotype with CD14^+ HLA-DR^- monocytes (Fig. 2A). Most of
the myeloid markers and surface activation markers such as CD11b,
CD16, CD33, CD80, and PD-L1 were expressed at similar levels in both populations. The CD14+HLA-DR−/low MDSCs from CHB patients, similar to CD14+HLA-DR+ cells, expressed positive CD11b and CD33. CD86 expression was rather decreased on CD14+HLA-DR−/low MDSCs, compared with CD14+HLA-DR+ monocytes. Notably, high expression of PD-1 was detected on MDSCs, but not on CD14+HLA-DR+ monocytes (Fig. 2B, bottom panel). Furthermore, we observed a significant positive correlation between MDSC frequency and PD-1 expression on total CD14+ monocytes (r = 0.631, p = 0.002). Increased PD-1 expression on total CD14+ monocytes was associated with increased percentage of CD14+HLA-DR−/low MDSCs in CHB patients (Fig. 2B, top panel). To further confirm the expression of PD-1 on MDSCs, Western blotting was used to determine the expression of PD-1. The results demonstrated that upregulation of PD-1 expression in MDSCs was detected, compared with that of CD14+HLA-DR+ monocytes (Supplemental Fig. 2). The data were consistent with those of FACS analysis.

We next examined the production of cytokines including IL-1β, IL-6, IL-10, and TNF-α in subsets of CD14+ cells under LPS stimulation in vitro (Fig. 2C, 2D). We found that CD14+HLA-DR−/low MDSCs from CHB patients expressed statistically significantly more IL-10 than did CD14+HLA-DR+ cells. However, MDSCs seemed to have decreased secretion of IL-1β, IL-6, and TNF-α compared with CD14+HLA-DR+ cells in these CHB patients. The similar results also were observed in the cell culture supernatants by cytokine bead array (Supplemental Fig. 3).

MDSCs in relationship to clinical parameters

To analyze whether the frequency of MDSCs are correlated with serum ALT levels and HBV DNA load, IA patients were divided into two groups based on their serum HBV DNA load or ALT level, respectively. As shown in Fig. 3A, patients with serum HBV DNA load of >10⁷ IU/ml had a higher mean percentage of circulating MDSCs than did patients with a serum HBV DNA load of <10⁷ IU/ml. However, patients with a serum ALT level of >200 U/l had a lower frequency of these cells, compared with subjects with an ALT level of <200 U/l (Fig. 3B). Interestingly, Spearman rank correlation analysis also showed a positive correlation between frequency of MDSCs and the viral load (Fig. 3A) but a negative relationship between frequency of MDSCs and ALT levels (Fig. 3B). These results suggested that increased circulating MDSCs may be associated with a negative immune response leading to poor viral clearance and, in contrast, contribute to protect liver inflammation injury. Additionally, we found that the percentage of this population was not correlated with HBeAg or HBsAg levels.

The relationship between intrahepatic MDSCs and liver HAI, including grading and staging, was further analyzed. The results showed that IA patients with HAI grade 1 had a higher frequency of MDSCs in the liver than did patients with HAI grades 2–4 (Fig. 3C), whereas no such difference was observed between HAI staging 1 and staging 2–4 groups (Fig. 3D), further suggesting these cells are negatively related to necroinflammatory activity, but not to fibrosis alteration.

MDSCs suppress cytolytic molecule release and production of CD8+ T cells in CHB patients

It is well known that hepatocyte injury in CHB patients results from the killing capability of CD8+ T cells. In this study, degranulation of cytolytic molecules by CD8+ T cells was measured by detecting the expression of the perforin-release marker, CD107a. After 6 h
of anti-CD3/CD28 stimulation, CHB patients showed a significant decrease of CD107a expression in CD8+ T cells compared with normal controls (Fig. 4A). Dynamic analysis showed that the CD107a expression was much lower in HBC patients than in normal controls after stimulation (Fig. 4B). When MDSCs were depleted, CD107a expression was restored significantly (Fig. 4B). Degranulation analysis showed that CHB patients with a serum ALT level of $\geq 200$ U/l had lower levels of GrB and perforin, compared with subjects with an ALT level of $> 200$ U/l (Fig. 4C).

Production of GrB and perforin in CD8+ T cells of CHB patients

FIGURE 3. Correlation analysis between the frequency of MDSCs and clinical parameters. (A and B) The significance of difference between this subset of monocyte frequency and serum HBV DNA (A) or ALT level (B) was analyzed using a nonparametric Mann–Whitney U test. The correlation between MDSC frequency and serum HBV DNA load or ALT level was analyzed using Spearman correlation. (C and D) Correlation analysis between the frequency of intrahepatic MDSCs in CHB and histological activity index, including grading (C) and staging (D). G, grading; S, staging.

FIGURE 4. MDSCs suppress degranulation of CD8+ T cells of CHB patients. (A) The degranulation marker, that is, CD107a expression on CD8+ T cells of CHB patients, was compared with normal controls after 6 h of anti-CD3/CD28 stimulation. (B) Dynamic analysis of CD107a expression on CD8+ T cells, as well as the effect of depletion of MDSCs from PBMCs of CHB patients on CD107a expression. (C) Levels of GrB and perforin production by CD8+ T cells in CHB patients with various ALT levels. (D and E) Depletion of MDSCs from PBMCs restores the production of GrB and perforin in CD8+ T cells. Means ± SD are shown. *$p < 0.05$, **$p < 0.01$. 

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increased obviously in MDSC-depleted PBMCs (Fig. 4D, 4E). Overall, our findings suggest that MDSCs may suppress CD8⁺ T cell killing capability by inhibiting the release and production of GrB and perforin, which contributes to the improvement of liver inflammation.

**MDSCs suppress CD8⁺ T cell response in CHB patients**

For further functional analysis, we tested whether MDSCs from CHB patients could suppress autologous CD8⁺ T cell responses. Sorted MDSCs were added at different ratios to autologous PBMCs. We detected that MDSCs suppressed the proliferation and IFN-γ production of autologous CD8⁺ T cells in a dose-dependent manner. Moreover, depletion of these cells from PBMCs of CHB patients resulted in a significantly stronger proliferation upon stimulation with anti-CD3/CD28 or higher IFN-γ production after stimulation with PMA compared with PBMCs (Fig. 5A, 5B). Furthermore, we observed that these cells inhibited the HBcAg-specific proliferation of CD8⁺ T cells in a dose-dependent manner, and autologous CD8⁺ T cell response was enhanced when these cells were depleted from PBMCs (Fig. 5C). Moreover, Transwell assays showed that the suppressive activity of these cells was mediated by soluble factors and was not cell contact–dependent (Fig. 5). We also evaluated the immunosuppressive activities of CD14⁺HLA-DR⁺ monocytes on autologous T cell proliferation and IFN-γ production. As expected, CD14⁺HLA-DR⁺ monocytes failed to inhibit CD8⁺ T cell proliferation or IFN-γ production in autologous PBMCs even at a 1:1 ratio (data not shown). Thus, the suppressive function was restricted to the HLA⁺DR⁻low population.

**PD-1–induced IL-10 production is responsible for MDSC-mediated suppression of T cell response**

We further investigated the underlying mechanism by which MDSCs suppress CD8⁺ T cell response. Although inducible NO synthase (iNOS) and arginase have been linked to MDSC activity of immunosuppression, in the current study we could not detect any arginase expression or activity in MDSCs from CHB patients, thereby excluding a role of arginase and iNOS metabolism products (Fig. 6A). Additionally, no obvious difference was found for the expression of p47phox and gp91phox, the most important subunits of the NADPH oxidase complex responsible for ROS production, between CHB patients and healthy controls (Fig. 6A). However, the level of IL-10 transcription was significantly higher in CHB MDSCs compared with levels of those in healthy controls (Fig. 6A), consistent with the results in Fig. 2C. The mRNA expression of TGF-β also was not detected in MDSCs. Furthermore, suppression of T cell response, including T cell proliferation and IFN-γ production, was prevented by treatment with IL-10–specific mAbs (Fig. 6B, 6C). However, inhibition of arginase, iNOS, and ROS had no observed effect on MDSC-mediated suppression of CD8⁺ T cell response (data not shown). Interestingly, we also observed similar results when we...

**FIGURE 5.** MDSCs inhibit proliferation and cytokine production of autologous CD8⁺ T cells. (A) Effect of MDSCs on CD8⁺ T cell proliferation. CFSE-labeled PBMCs-MDSCs, PBMCs, and PBMCs plus MDSCs in normal 96-well at a ratio of 10:1 and 1:1, or in Transwell, were cocultured with anti-CD3/CD28 stimulation for 5 d, followed by cytometric analysis of the cell division cycle. PBMC | +MDSC indicates results from the Transwell assay. Data were from five representative CHB patients. (B) Effect of MDSCs on IFN-γ production by CD8⁺ T cells. PBMCs-MDSCs, PBMCs, and PBMCs plus MDSCs in normal 96-well at a ratio of 10:1 and 1:1, or in Transwells, were cocultured with PMA/ionomycin stimulation for 6 h. IFN-γ was detected by intracellular cytokine staining. (C) CFSE-labeled cells described above were cocultured with HBcAg stimulation for 5 d, followed by cytometric analysis of the cell division cycle. Data were from at least five CHB individuals. Means ± SD are shown. *p < 0.05, **p < 0.01.
added the neutralizing Ab for PD-L1 to cell cultures. Moreover, after pretreatment with anti–PD-L1 mAb for these cell cultures, addition of anti–IL-10 mAb could not effectively restore T cell proliferation and IFN-γ production (Fig. 6B, 6C). These results strongly suggest that the PD-1 signaling might play an important role in inducing IL-10 production by MDSCs.

We further investigated whether triggering of PD-1 can induce IL-10 production by MDSCs. Stimulation of MDSCs with a PD-1–specific agonist induced a 10-fold increase in IL-10 production as compared with control IgG (Fig. 6D). Moreover, MDSCs triggered via anti–PD-1 Ab produced IL-10 as early as 6 h after treatment and reached peak concentration at 24 h (Fig. 6F). In contrast, we did not detect IL-10 production upon PD-1 triggering of CD3− and CD28-preactivated T cells and CD14+HLA-DR+ cells (data not shown), suggesting that IL-10 production was from PD-1–triggered MDSCs and that it mediated suppression of T cell response.

To further confirm the impact of PD-1 interaction with PD-L1 or PD-L2 on IL-10 production by MDSCs. We transduced COS-7 cells with pcDNA3.1 encoding GFP (COS-mock cells), PD-L1 (COS–PD-L1 cells), and PD-L2 (COS–PD-L2 cells). MDSCs produced more IL-10 when cocultured with COS–PD-L1 cells than did MDSCs cocultured with COS-mock cells. Addition of anti–PD-L1 mAb into the coculture inhibit the IL-10 production was by MDSCs. Additionally, MDSCs cocultured with COS–PD-L2 did not produce much IL-10 (Supplemental Fig. 4). Overall, these data demonstrated that PD-1/PD-L1 signaling was responsible for IL-10 production in MDSCs.

**Discussion**

There has been rapid progress in the understanding of the contribution of MDSCs in tumor progression and antitumor immune response (32). However, the immunological and clinical significances of MDSCs in viral diseases are far from clear. In this study, we have identified, to our knowledge for the first time, a novel subset of monocytes from CHB patients that are capable of suppressing T cell responses. These cells, which were CD14+, did not express molecules that are associated with activation or costimulation, such as HLA-DR or CD80. The suppressive monocyte population has been identified in the microenvironment of tumor systems (14, 15), which was also named “MDSCs.” In the current study, we found a significantly higher proportion of this subset in CHB patients compared with healthy controls. We also detected that increased MDSCs were positively correlated with HBV viral load, but negatively correlated with liver damage, suggesting that increased MDSCs could be associated with disease progression. Furthermore, our results indicate that these cells suppress IFN-γ secretion and proliferation of HBV-specific T cells in a cell contact–independent fashion, which required production of IL-10. Overall, our data provide a novel mechanism of viral escape from effective immune response, and it could also be a response of the host to avoid further organ damage.

Two previous studies have characterized the peripheral MDSCs in chronic hepatitis C patients and suggested that MDSCs may play a role in the pathogenesis of viral infections (18–20). However, in contrast with these studies, Nonnenmann and colleagues (33) showed that MDSCs in peripheral blood are not of significance for immune dysfunction in chronic hepatitis C. We postulated that the differences in frequency and suppressive capacity of MDSCs might partly result from different MDSC subsets and various HCV
effects of MDSCs on CD8+ T cell proliferation and IFN-

gentrabial expression of HLA-DR in a cohort of CHB patients, in agreement

with previous studies in HBV transgenic mice and HIV patients (21, 22, 34). Interestingly, the presence of MDSCs was associated with increased PD-1 expression, suggesting that the immunosuppressive phenotype of MDSCs in CHB patients is CD14+HLA-

DR−/lowPD-1−. The persistence of viral infections has been found to be associ-

ated with diminished CD8+ T cell number and function in both humans and mice (35). Several studies have demonstrated that the cellular immune response to HBcAg is generally low to undetect-

able in chronic HBV patients (36, 37). Thus, we hypothesized that MDSCs contribute to the suppression of immune response in these patients. Our results revealed that MDSCs obtained from CHB patients were able to inhibit autologous T cell proliferation and production of IFN-γ in vitro. Furthermore, we investigated T cell cytokotoxic dysfunction in CHB patients by measuring CD107a, a surrogate marker for cytotytic activity, and their granule compo-

nants, notably perforin and GrB. The levels of CD107a, perforin, and GrB were also significantly inhibited by MDSCs. These data suggest that MDSCs may impair CD8+ T cell function by suppress-

ing T cell proliferation, activation, and degranulation in CHB patients, which is consistent with the negative correlation between MDSCs and liver injury.

In most tumor models, suppression is caused by a cell–cell contact-dependent mechanism (8, 9, 29), yet the precise mecha-

nism of suppression in CHB is not known. Pervious studies have shown that increased arginase-1 or ROS generation could explain the mechanism of suppressive MDSCs in chronic virus infection (19–21). However, our study supports that upregulation of IL-10 represents the major mechanism for suppressive MDSCs in CHB patients, similar to previous reports for several other human dis-

cases (27, 38, 39). These cells could spontaneously secrete bio-

active IL-10 ex vivo, a feature undetectable in healthy control MDSCs. Thus, inhibition of either one could restore T cell sup-

pression. IL-10 is essential and not redundant to the suppressive function of MDSCs (27, 40, 41). Indeed, we observed that neu-

ralizing Abs to PD-L1 or IL-10 similarly reversed the inhibitory effects of MDSCs on CD8+ T cell proliferation and IFN-γ pro-

duction. Recently, the relationship of PD-1 level and IL-10 produc-

tion by monocytes has been described in patients with HIV infection (42). In the current study, we found that PD-1 and PD-L1 were expressed in MDSCs. Thus, we postulated that IL-10 produc-

tion by MDSCs was induced by PD-1/PD-L1 interaction. As expected, triggering of PD-1 significantly induced IL-10 produc-
	
tion. Taken together, our data provide evidence that upregulation of PD-1 on MDSCs during HBV infection may lead to an impaired immune system that is incapable of responding with an appro-

riate immune response against HBV.

In this study, we cannot exclude the possibility that the increased percentage of MDSCs was associated with liver inflammation, because the causal relationship between inflammation and MDSC accumula-

tion has been well documented under other pathological conditions (43, 44). However, the strong correlation between MDSCs and HBV viral load suggests that the high number of MDSCs capable of suppressing HBV-specific T cell responses is most likely caused by the viral infection itself. It has been suggested that MDSCs can be mobilized, expanded, or differen-

tially harbored by virus itself or its coding proteins, including HCV, HIV, and lymphocytic choriomeningitis virus (20–22, 45). Chronic HBV-infected patients are known to have large amounts of HBV Ags in their peripheral blood. An additional factor that may contribute to the increased percentage of MDSCs is the tol-

erogenic environment of the liver, the primary site of HBV replication. The detailed mechanisms of the generation and accumu-
luration of MDSCs in CHB are being investigated in our group.

In conclusion, to our knowledge, the current study demonstrates for the first time the importance of MDSCs in chronic HBV in-

fection. We showed that patients with chronic HBV infection contained a higher percentage of MDSCs in their peripheral blood compared with healthy controls. These MDSCs could contribute to persistence of HBV infection by impairing HBV-specific immune response, but also inhibited serious inflammatory response resulting in further liver damage. Our findings provide new insights into the mechanisms of MDSCs in CHB patients. Our study might support the potential of targeting MDSCs for developing effective immunothe-

rapy for HBV infection.

Acknowledgments

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Disclosures

The authors have no financial conflicts of interest.

References


10. Filippazzi, P., R. Valenti, V. Huber, L. Pilla, P. Canese, M. Iero, C. Castelli, L. Bonini, G. Pariani, and L. Rovinelli. 2007. Identification of a new subset of myeloid suppressor cells in peripheral blood of melanoma patients with modula-
	


induce IDOhi CD14+HLA-DRlo myeloid-derived suppressor cells that inhibit T-cell responses and promote Treg. Blood 124: 750–760.


Supplementary Figure 1  Frequencies of Lin^−HLA-DR^−CD33^+ cells in CHB (n=21) as compared to HC (n=14) PBMC. Bars indicate mean ± SD.
The expression of PD-1 on MDSCs was determined by western blotting. MDSCs and CD14⁺HLA-DR⁺ monocytes derived from three patients were sorted, and the cell samples were used to determine the PD-1 expression by western blotting.
Supplementary Figure 3  Cytometric Bead Assays were performed to quantify IL-1β, IL-6, and TNF-α production in the supernatants. Bars indicate mean ± SD.
Supplementary Figure 4  IL-10 expression was measured by ELISA at 48 h after MDSCs incubation with PD-L1-expressing (in the presence or absence of antibody to PD-L1), PD-L2-expressing or Mock (empty vector)-expressing Cos-7 cells at a ratio of 1:4.