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# B7-H1 Up-Regulation on Myeloid Dendritic Cells Significantly Suppresses T Cell Immune Function in Patients with Chronic Hepatitis B<sup>1</sup>

Liangen Chen,<sup>2\*</sup> Zheng Zhang,<sup>2\*</sup> Weiwei Chen,<sup>\*</sup> Zhidong Zhang,<sup>†</sup> Yonggang Li,<sup>\*</sup> Ming Shi,<sup>\*</sup> Jiyuan Zhang,<sup>\*</sup> Lieping Chen,<sup>†‡</sup> Shengdian Wang,<sup>3†</sup> and Fu-Sheng Wang<sup>3\*</sup>

Although dysfunctional dendritic cells contribute to inadequate adaptive immunity in chronic hepatitis B (CHB), underlying molecular mechanisms remain largely undefined. In this study, we examined B7-H1 expression on circulating myeloid dendritic cells (mDCs) in 46 CHB patients, 10 autoimmune hepatitis patients, and 10 healthy subjects as control. We found that B7-H1 expression is significantly up-regulated on circulating mDCs of CHB and autoimmune hepatitis patients compared with healthy individuals. The B7-H1 up-regulation was significantly correlated with an elevation of serum alanine aminotransaminase levels and plasma viral load. In addition, *in vitro*, both IFN- $\alpha$  and IFN- $\gamma$  could strongly stimulate mDCs to express B7-H1. More importantly, elevated B7-H1 expression is also closely associated with the suppression of T cell immune function. *In vitro* blockade of B7-H1 signaling could not only down-regulate IL-10 and up-regulate IL-12 production by mDCs, but also enhance mDC-mediated allostimulatory capacity and cytokine production of T cells. Blockade of B7-H1 signaling could improve hepatitis B c Ag-pulsed monocyte-derived DC-induced IFN- $\gamma$  production by autologous hepatitis B virus-specific T cells. These new findings suggested that chronic inflammation may contribute to B7-H1 up-regulation on mDCs in CHB patients, which potentially cause defective hepatitis B virus-specific T cell function and viral persistence. Our findings further support the notion that the blockade of B7-H1 may represent a novel therapeutic approach for this disease. *The Journal of Immunology*, 2007, 178: 6634–6641.

The host immune response to the hepatitis B virus (HBV)<sup>4</sup> is a critical factor in determining the outcome of HBV infection (1). During the natural course of HBV infection, patients with self-limited acute hepatitis B may develop strong virus-specific CD8 and CD4 T cell responses, although these T cell immune responses against the virus are insufficient to control infection in chronic hepatitis B (CHB) carriers (2–4). The mechanisms underlying this defect of specific T cell immunity have not yet been fully elucidated. It is possible that deletion (apoptosis) and functional tolerance (exhaustion, anergy, and dysregulation of lymphokine production) of specific T cells contribute to hypore-

sponsiveness in hosts who are continuously exposed to high levels of viral Ags (5). In addition, CD4<sup>+</sup>CD25<sup>+</sup>Foxp3<sup>+</sup> regulatory T cells during chronic HBV infection have been proposed to not only modulate effectors of the immune response to HBV infection, but also to influence disease progression in patients with hepatitis B (6, 7). Understanding the mechanisms underlying T cell hyporesponsiveness will have a profound influence on the establishment of an immune therapeutic regimen to break immunological tolerance and thereby terminate persistent viral infection.

Dendritic cells (DCs) represent the most potent APCs and, thus, play an important role in the initiation and maintenance of specific T cell immunity (8, 9). Two distinct subpopulations of DCs, myeloid DCs (mDCs) and plasmacytoid DCs, have been identified in humans. mDCs have the capacity to produce large amounts of IL-12 following bacterial or viral infection, whereas plasmacytoid DCs selectively produce high levels of type I IFNs following viral infection. In particular, mDCs play a pivotal role in adaptive immunity by activating naive T cells (8, 9). In animal models (10) and human clinical trials (11, 12), adoptively transferred mDCs expressing viral Ags could mediate substantial antiviral T cell immunity. Recent studies have shown that defects in DC function could be an important factor in the host-specific T cell immune tolerance to viral infection (13), and therefore may facilitate viral persistence in HIV-1- (14), hepatitis C virus- (HCV) (15, 16), and HBV-infected patients (17–19). However, the molecular mechanisms by which dysfunctional mDCs mediate the inhibition of T cell immune responses remain elusive, particularly in chronic HBV infection.

B7-H1 (also called CD274 or programmed death (PD)-L1) is a cell surface glycoprotein belonging to the B7 family of costimulatory molecules (20). B7-H1 is expressed widely by activated DCs, macrophages, T cells, B cells, and monocytes, as well as by nonlymphoid tissues. B7-H1 does not interact with CD28,

\*Research Center of Biological Therapy, Beijing 302 Hospital, Beijing, China; †Center for Infection and Immunity, National Laboratory of Biomacromolecules, Institute of Biophysics, Chinese Academy of Sciences, China; and ‡Department of Dermatology, Department of Oncology, and the Institute for Cell Engineering, Johns Hopkins University School of Medicine, Baltimore, MD 21231

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<sup>2</sup> L.C. and Z.Z. contributed equally to this work.

<sup>3</sup> Address correspondence and reprint requests to Dr. Fu-Sheng Wang, Research Center for Biological Therapy, Beijing Institute of Infectious Diseases, Beijing 302 Hospital, 100 Xi Si Huan Middle Road, Beijing, China or Dr. Sheng-Dian Wang, Center for Infection and Immunity, National Laboratory of Biomacromolecules, Institute of Biophysics, Chinese Academy of Sciences, 15 Datun Road, Chaoyang District, Beijing, China. E-mail addresses: fswang@public.bta.net.cn or sdwang@moon.ibp.ac.cn

<sup>4</sup> Abbreviations used in this paper: HBV, hepatitis B virus; CHB, chronic hepatitis B; DC, dendritic cell; mDC, myeloid DC; HCV, hepatitis C virus; PD, programmed death; ALT, alanine aminotransferase; IT, immune tolerant; IA, immune active; HC, healthy control; AIH, autoimmune hepatitis; MoDC, monocyte-derived DC; HBcAg, hepatitis B c Ag.

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Table I. Characteristics of the populations enrolled in the study

	Patient			
	HC	IT	IA	AIH
Cases	10	11	35	10
Age (years)	30 (24–38)	28 (21–35)	34 (18–54)	40 (24–66)
Gender (male/female)	7/3	9/2	25/10	2/8
Serum HBV DNA (copies/ml)	NA <sup>a</sup>	$1.8 \times 10^7$ ( $1.0 \times 10^6$ – $1.6 \times 10^8$ )	$9.7 \times 10^5$ ( $3.4 \times 10^3$ – $2.14 \times 10^9$ )*	NA <sup>a</sup>
Serum ALT (U/L)	18 (7–34)	24 (15–38)	85 (41–558)*	55 (20–201)*

<sup>a</sup> NA, Not applicable; data are shown as median and range.

\*,  $p < 0.05$ , compared with IT group.

CTLA-4, or ICOS, but has been shown to bind to PD-1, a molecule belonging to the Ig superfamily (21). B7-H1 binding to PD-1 could deliver a coinhibitory signal to the T cells, which has been demonstrated to be involved in the regulation of T cell activation and tolerance (22–25). Several studies have addressed the key roles of high-level endogenous B7-H1 expression in controlling antitumor (26, 27) and antiviral immune responses (28–36). In particular, a recent study reported that the B7-H1/PD-1 pathway could result in viral-specific CD8 T cell functional exhaustion, whereas blockade of this pathway could restore viral-specific CD8 T cell function and reduce viral burden in the chronic infected mouse model of lymphocytic choriomeningitis virus infection (29). Quite recently, increased B7-H1/PD-1 signaling has been implicated in viral-specific CD8 T cell exhaustion in HIV-1 (30–33), HBV (34, 35), and HCV infection (36) and was associated with disease progression. However, it is unknown whether B7-H1 was up-regulated on mDCs in CHB patients and, if so, how B7-H1 up-regulation contributes to mDC-mediated inhibition of T cell immune responses in patients with CHB.

In the present study, we found that B7-H1 was substantially up-regulated on mDCs from patients with CHB and autoimmune hepatitis, and significantly suppressed mDC-mediated antiviral T cell immune responses during chronic HBV infection. The blockade of B7-H1 enhanced mDC-mediated T cell immune responses in CHB patients. Our report suggests that chronic inflammation may induce B7-H1 up-regulation, which subsequently mediates HBV-specific T cell suppression and may be responsible for the chronicity of hepatitis B. These findings provide important insights that will promote the development of a DC-based immunotherapeutic strategy.

## Materials and Methods

### Subjects

This study examined 46 adults with chronic HBV infection (34 male, 12 female; median age, 32.8 years (range, 18.0–54.2 years)), seropositive for hepatitis B surface Ag and hepatitis B e Ag, for at least 12 mo. All patients were admitted to hospitalization in our unit, Beijing 302 Hospital, from August 2005 to August 2006. No patients received anti-HBV agents or steroids 6 mo before sampling. Concurrence of HCV, hepatitis D virus, hepatitis G virus, and HIV infections were excluded from enrolled individuals. The study protocol was approved by the Ethics Committee of our unit, and informed consent was obtained from each subject.

The patients were assigned to two distinct groups, with respect to plasma HBV DNA loads and alanine aminotransferase (ALT) levels according to our previous reports (37). The first group ( $n = 11$ ) was defined as immune-tolerant (IT) adults with highly active HBV replication, but normal ALT levels (normal range: 10–40 U/L). The second group ( $n = 35$ ) was immune-active (IA) adults with active HBV replication and significantly elevated serum ALT levels. Baseline clinical data are shown in Table I. For comparison, 10 uninfected healthy controls (HC) and 10 patients with autoimmune hepatitis (AIH) were age and sex matched to the enrolled patient cohorts as controls.

### Preparation of mDCs and T cells

PBMCs were isolated by Ficoll-Hypaque density gradient centrifugation either from 20 ml of heparinized blood or 2 ml of leukopheresis-derived PBMC-enriched samples. CD11c<sup>+</sup> DCs were purified from PBMCs by CD19-negative selection, followed by CD1c-positive selection using the CD1c (BDCA-1) Dendritic Cell Isolation Kit (Miltenyi Biotec) with a Midi MACS separator unit. CD3<sup>+</sup> T cells were isolated from PBMCs by positive selection using a CD3 Positive Isolation Kit (Miltenyi Biotec). CD4<sup>+</sup> and CD8<sup>+</sup> T cells were also isolated from PBMCs by positive selection using the CD4 and CD8 Positive Isolation Kit (DynaL Biotec). All cell separations were performed according to the manufacturer's instructions. Isolated populations of mDCs, CD3<sup>+</sup>, CD4<sup>+</sup>, and CD8<sup>+</sup> T cells were >95% in purity. Unless otherwise stated, freshly isolated cells were cultured in complete RPMI 1640 medium containing 10% FCS, 100 U/ml penicillin, and 100  $\mu$ g/ml streptomycin.

### mDC culture and B7-H1 expression

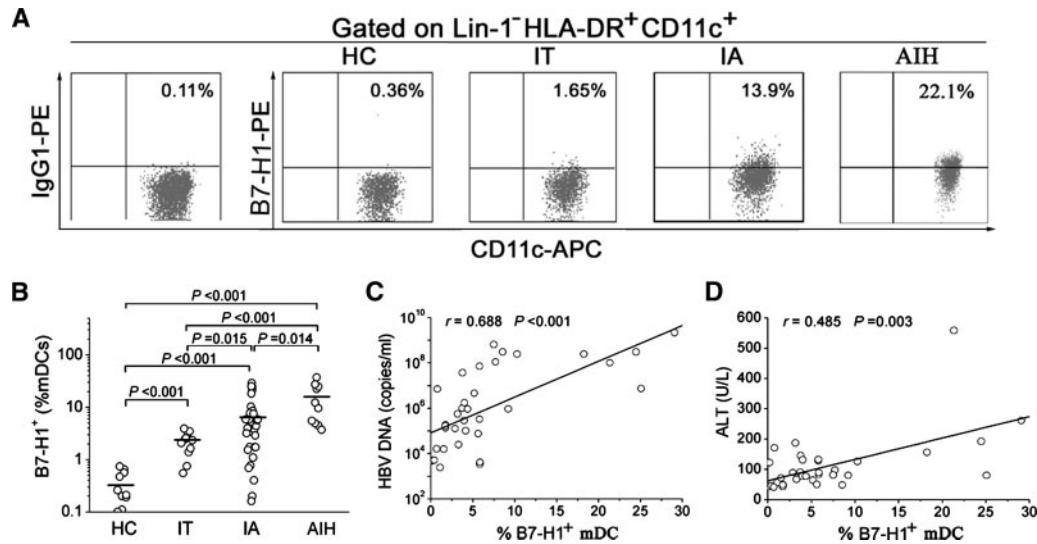
CD11c<sup>+</sup> DCs were purified from the PBMCs of three healthy subjects as described as above. These isolated mDCs were incubated in a 96-well plate in RPMI 1640 medium containing 10% FCS only or with IFN- $\alpha$  (10,000 U/ml) or IFN- $\gamma$  (1,000 U/ml). Cells were collected at 0, 3, 6, 9, 12, 24, and 48 h of in vitro cell culture. The B7-H1 expression was analyzed using a FACSCalibur (BD Biosciences).

### Preparation of monocyte-derived DCs (MoDCs)

MoDCs were prepared from blood monocytes according to previously established protocols, with some modifications (38). In brief, PBMCs were suspended at  $5 \times 10^6$  cells/ml in 6-well plates in serum-free AIM-V medium (Invitrogen Life Technologies). After incubation for 1 h at 37°C in a 5% CO<sub>2</sub>, humidified atmosphere, nonadherent cells were gently removed, and the remaining cells were incubated in medium supplemented with GM-CSF (800 U/ml) and IL-4 (1000 U/ml) (PeproTech). Cells were fed on day 2 with fresh medium containing IL-4 and GM-CSF. On day 6, hepatitis B e Ag (HBcAg; Kangtai Biological) was added at a final concentration of 20  $\mu$ g/ml for a 24-h incubation. Then, MoDCs were matured by incubation with IL-1 $\beta$ , TNF- $\alpha$ , IL-6 (all at 1000 U/ml; PeproTech), and PGE<sub>2</sub> (10 ng/ml; PeproTech) for another 24 h. Mature MoDCs were collected for phenotypic analysis or other experiments, and the supernatant was collected for future cytokine measurement.

### Flow cytometric analysis

All Abs were purchased from BD Biosciences, except anti-B7-H1-PE (clone MIH4) and its isotype control Ab IgG1-PE (eBioscience). 5H1, a B7-H1-neutralizing mAb, has been described previously (39). B7-H1 expression on circulating mDCs was measured using 0.4 ml of fresh heparinized peripheral blood, incubated with a mixture of Abs (lineage-1-FITC, HLA-DR-PerCP, CD11c-allophycocyanin, and B7-H1-PE, or the corresponding PE-conjugated isotype controls) for 20 min at 4°C. The cells were then lysed with FACS lysing solution (BD Pharmingen) to remove RBC, fixed, and analyzed by four-color flow cytometry on a FACSCalibur (BD Biosciences). For intracellular cytokine staining, isolated T cells were first incubated with anti-CD8-PerCP and anti-CD3-allophycocyanin. These cells were further treated with the permeabilization agent Cytofix/Cytoperm (BD Pharmingen) according to the manufacturer's instructions and then intracellularly stained with anti-IFN- $\gamma$  and anti-IL-4 Abs at room temperature for 20 min in the dark. After washing twice with Cytoperm/Wash



**FIGURE 1.** B7-H1 expression is up-regulated on mDCs in CHB infection. **A**, Representative dot plots of B7-H1 staining for IA, IT, and AIH patients and HC. B7-H1 expression was determined by FACS gated on Lin<sup>-</sup>CD11c<sup>+</sup>HLA-DR<sup>+</sup> cells. Values in the *upper right quadrant* represent the percentage of mDCs that express B7-H1. **B**, B7-H1 expression on mDCs is significantly up-regulated in chronic HBV patients and AIH patients, as compared with HC. ○, One individual. Horizontal bars, Mean values. **C** and **D**, Correlation analysis between the levels of B7-H1 expression on mDCs and serum ALT levels (**C**) or plasma HBV load (**D**) in IA individuals. Solid line, Linear growth trend. Values of *p* are shown.

(BD Pharmingen), the cells were fixed in 1% paraformaldehyde, and four-color flow cytometric analysis was performed using a FACSCalibur instrument and CellQuest software (BD Biosciences).

#### Allogeneic MLR

Purified mDCs were matured for 24 h in 96-well flat-bottom culture plates (at  $1 \times 10^5$  cells/200  $\mu$ l) in culture medium containing poly(I:C) (50  $\mu$ g/ml; Amersham Biosciences). On the following day, mDCs were added at different concentrations (1/5, 1/10, 1/20, 1/40, and 1/80) into purified CD4 or CD8 T cells from a normal healthy volunteer ( $1 \times 10^5$  cells/200  $\mu$ l) in the presence of 10  $\mu$ g/ml anti-B7-H1 or control Ig for 5 days. T cell proliferation was assessed by the incorporation of [<sup>3</sup>H]thymidine (Amersham Biosciences); 1  $\mu$ Ci/well was added, and cultures were harvested 18 h later. Before measurement of incorporated [<sup>3</sup>H]thymidine, part of the culture supernatants was collected for cytokine detection.

#### IFN- $\gamma$ ELISPOT assay

Freshly isolated T cells were plated ( $1 \times 10^5$  cells/well) and cocultured with graduated numbers of autologous HBcAg-pulsed mDCs in the presence of 10  $\mu$ g/ml anti-B7-H1 or control Ig for 48 h. T cells incubated in medium with or without PMA (50 ng/ml; Sigma-Aldrich) were used as positive and negative controls, respectively. A 96-well ELISPOT assay (U-CyTech Biosciences) for IFN- $\gamma$  production was performed and analyzed according to the manufacturer's instructions. The number of spot-forming units was determined with computer-assisted image analysis software (ChampSpot; Saizhi). A response was defined in this study as an Ag-specific IFN- $\gamma$ -producing frequency of 10 spots/well, and 3 SDs greater than the mean frequency in the absence of Ag. Results were expressed as spot-forming units per  $10^5$  PBMC.

#### ELISA

The concentrations of IL-12, IL-2, IL-10, and IFN- $\gamma$  in the culture supernatants were measured with ELISA kits (eBioscience). Absorbance was measured on an automatic plate reader. The sensitivity of the assays was 10 pg/ml.

#### Statistical analysis

All data were analyzed using SPSS version 13.0 for Windows (SPSS). For multiple comparisons, the Kruskal-Wallis H nonparametric test was applied. Statistical difference between the two groups was determined by applying the Mann-Whitney *U* nonparametric test. A paired *t* test was used to show the effect of treatment with Abs to B7-H1 on T cell function. Spearman correlation analysis was performed between the frequency of B7-H1 expressing mDCs and other parameters. A *p* < 0.05 was considered as a significant difference.

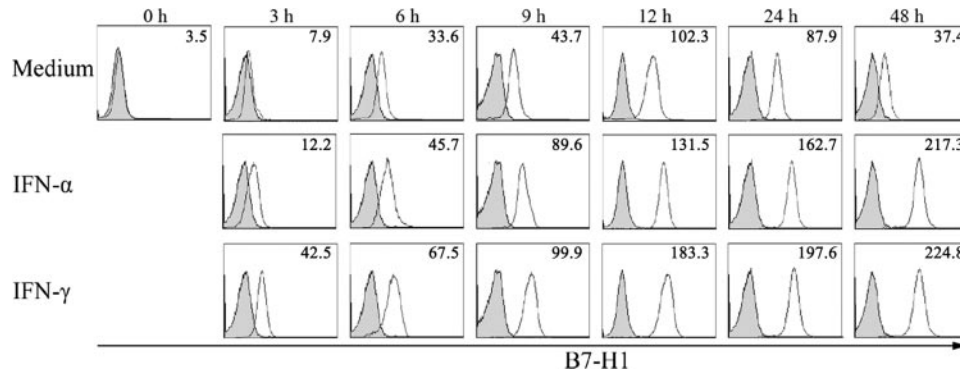
## Results

### *The expression of B7-H1 on mDCs was significantly up-regulated in patients with chronic HBV infection and AIH*

We first detected B7-H1 expression on mDCs from HBV-infected subjects, AIH patients, and healthy controls (Fig. 1A). We found that B7-H1 expression on mDCs from HBV-infected patients was significantly increased (*p* < 0.001), in particular for the mDCs of IA patients, which expressed a higher level of B7-H1 than that of IT individuals (*p* < 0.05; Fig. 1B). In addition, we found that AIH patients exhibited the highest levels of B7-H1 expression on mDCs among these four groups (all *p* < 0.05; Fig. 1B). Correlation analysis in IA patients revealed that there was a significant, positive correlation between B7-H1 expression on mDCs and serum plasma HBV load (*r* = 0.688, *p* < 0.05; Fig. 1C) and ALT levels (*r* = 0.485, *p* < 0.05; Fig. 1D). These data indicate that B7-H1 expression on mDCs is markedly up-regulated in CHB and AIH patients.

### *Both IFN- $\alpha$ and IFN- $\gamma$ induce in vitro B7-H1 expression on mDCs*

Although IFN- $\alpha$  and IFN- $\gamma$  have been shown to stimulate hepatocytes to express B7-H1 (40), whether these cytokines are required for up-regulation of B7-H1 on mDCs remains yet unknown. To highlight this question, we investigated the effects of IFN- $\alpha$  and IFN- $\gamma$  on B7-H1 expression on isolated mDCs from healthy subjects. We found that B7-H1 expression on mDCs exhibits a similar dynamic pattern in responses to IFN- $\alpha$  and IFN- $\gamma$  stimulation in vitro. Expression of the B7-H1 molecule can be detected on the surface of mDCs at 3 h and gradually increased to the peak level at 48 h after the onset of stimulation (Fig. 2). Compared with IFN- $\alpha$  stimulation, IFN- $\gamma$  could stimulate B7-H1 up-regulation on mDCs more efficiently during the former 24 h of in vitro culture. In contrast, mDCs cultured with medium only exhibited a slight and transitory up-regulation in B7-H1 expression. These data indicate that both IFN- $\alpha$  and IFN- $\gamma$ , in particular IFN- $\gamma$ , can stimulate mDCs to express B7-H1.



**FIGURE 2.** IFN- $\alpha$  and IFN- $\gamma$  can induce B7-H1 expression on mDCs in vitro. Representative analysis of B7-H1 expression on isolated mDCs from a HC. The isolated mDCs were incubated with medium only or in the presence of IFN- $\alpha$  or IFN- $\gamma$  in 96-well plates for 48 h. The stimulated cells were harvested at different time points, and B7-H1 expression on mDCs was analyzed using flow cytometry. Cells were gated on CD11c<sup>+</sup>HLA-DR<sup>+</sup> cells. Open histograms, Staining of B7-H1; filled histograms, staining of isotype controls. Numbers in histograms represent mean fluorescence intensity of B7-H1 expression. Data shown are representative of three independently performed experiments.

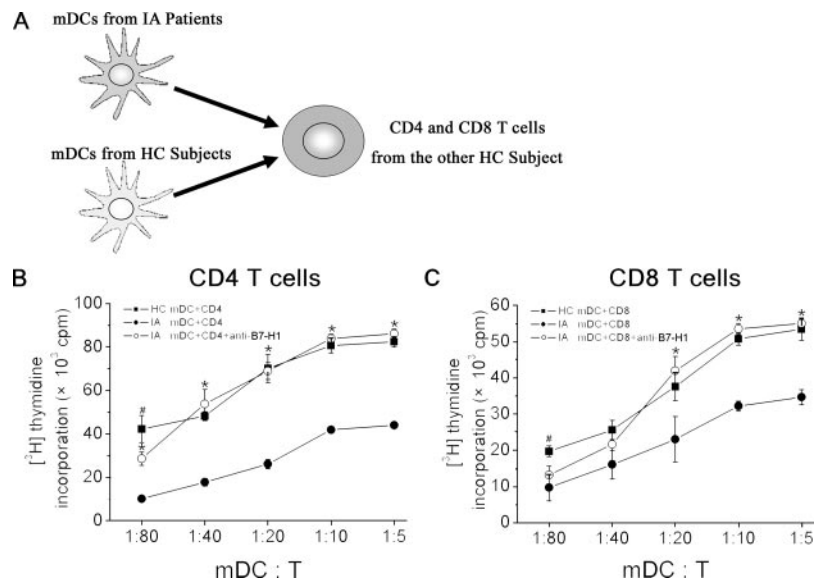
*Blockade of B7-H1 improves the allostimulatory capacity of mDCs from chronic HBV-infected patients*

We next found that the T cell-stimulating capacity of mDCs in an allogeneic MLR was affected in chronic IA patients. Mature mDCs from healthy subjects and IA patients were cocultured at different ratios with T cells of a healthy control (as the third party). Fig. 3 shows that mDCs of IA patients were less efficient in inducing both CD4 T cell (Fig. 3B) and CD8 T cell (Fig. 3C) proliferation at all ratios tested compared with mDCs isolated from healthy controls ( $p < 0.05$  for both). We further examined the effects of mDC-associated B7-H1 up-regulation on allogeneic T cell proliferation in the presence or absence of anti-B7-H1. We found that the poor capacity of mDCs to stimulate either CD4 T cell or CD8 T cell proliferations was fully restored by addition of anti-B7-H1 (5H1) mAb, leading to results similar to those found for normal mDCs (all  $p > 0.05$ ). To test whether anti-B7-H1 might block apoptotic signals for the T cells and further contribute to this elevated T cell proliferation phenomenon, we monitored T cell apoptosis in our MLR cultures using 7-aminoactinomycin D vs annexin V staining. We found that the number of annexin V<sup>+</sup> T cells in the absence or presence of anti-B7-H1 was only marginally altered in four experiments (data not show). These data indicate that B7-H1 up-regulation

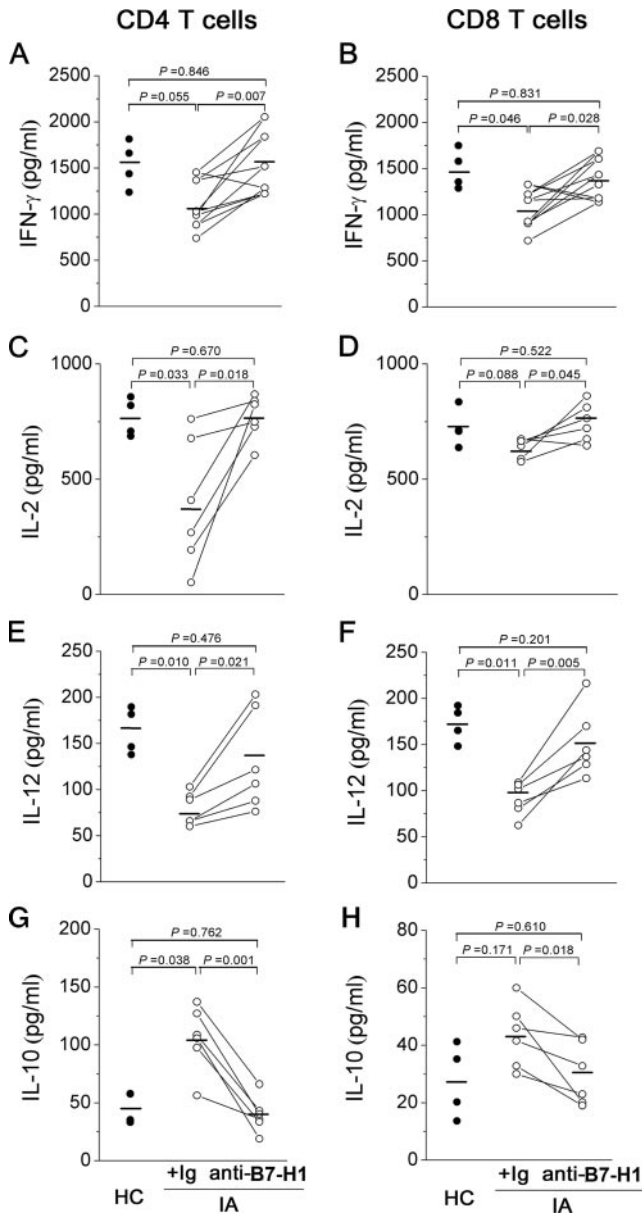
on mDCs appears to be responsible for the decreased proliferation of CD4 and CD8 T cells in chronic HBV-infected patients, while the blockade of B7-H1 can restore the allostimulatory capacity of mDCs in CHB patients.

*mDC-associated B7-H1 up-regulation is involved in suppression of cytokine production by T cells*

We also measured the production of type 1 effector cytokines including IFN- $\gamma$  and IL-2 in the coculture supernatants. Similar to the allostimulatory capacity, we found that the mDCs of CHB patients were less efficient in inducing IFN- $\gamma$  and IL-2 production by both CD4 (Fig. 4, A and C) and CD8 T cells (Fig. 4, B and D) than mDCs isolated from healthy controls ( $p < 0.05$ ). Furthermore, we found that upon addition of anti-B7-H1, the low-level production of both IFN- $\gamma$  and IL-2 by CD4 and CD8 T cells was both significantly increased at each mDC concentration (all  $p < 0.05$ ), yielding results similar to normal mDCs. Thus, B7-H1 up-regulation on mDCs appears to transmit inhibitory signals to T cells in chronic HBV-infected patients, resulting in a decrease of proliferation and cytokine production of T cells, while this inhibitory signal can be blocked by treatment with anti-B7-H1.

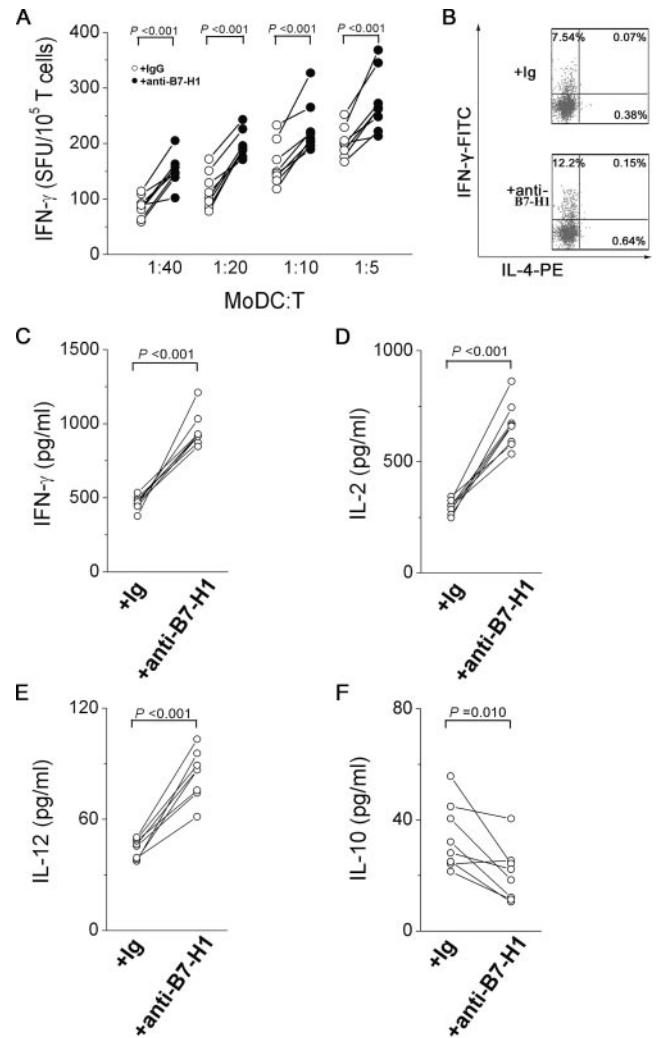


**FIGURE 3.** Blockade of B7-H1 can restore the T cell allostimulatory capacity of mDCs from IA individuals. **A**, Allostimulatory capacity of peripheral mDCs. mDCs from IA patients and HC were cultured at different numbers with T cells from a third-party healthy individual. After 5 days, the cells were pulsed for another 18 h with [<sup>3</sup>H]thymidine. **B** and **C**, mDCs of chronic HBV patients ( $n = 6$ ) showed a significantly reduced capacity to stimulate allogeneic CD4 (**B**) and CD8 T (**C**) cells at all ratios tested compared with healthy controls ( $n = 4$ ). The blockade B7-H1 signals significantly reversed the capacity to stimulate allogeneic CD4 (**B**) and CD8 T (**C**) cells of mDCs at all ratios. \*, Compared with addition of Ig; #, compared with healthy controls. Values of  $p$  are shown.



**FIGURE 4.** Blockade of B7-H1 can restore cytokine production by responsive cells in MLR. mDCs from IA patients and HC were cultured at different numbers with T cells from a third healthy individual. After 5 days, the cocultured supernatants were collected for ELISA. IFN- $\gamma$  (A and B), IL-2 (C and D), IL-12 (E and F), and IL-10 (G and H) were detected. mDCs of chronic HBV patients ( $n = 6$ ) showed a significantly reduced capacity to produce IL-12 and IL-10 and to stimulate allogeneic CD4 and CD8 T cells to produce IFN- $\gamma$  and IL-2 at a 1:10 ratio, compared with HC ( $n = 4$ ), whereas blockade B7-H1 signals significantly reversed the capacity to produce these cytokines. Values of  $p$  are shown.

To determine the mechanisms of the B7-H1 blockade in the modulation of allogeneic T cell responses, we measured the levels of IL-12 and IL-10 production in MLR culture supernatants. Using ELISA detection, we found that the IL-12 produced by mDCs in IA patients was significantly lower than in healthy controls ( $p < 0.05$ ; Fig. 4, E and F), whereas the Ab against B7-H1 significantly increased IL-12 production by these mDCs. On the contrary, IL-10 production in MLR culture supernatants was significantly higher for CHB patients than for healthy controls ( $p < 0.05$ ), and the blocking B7-H1 signal significantly decreased IL-10 production ( $p < 0.05$ ; Fig. 4, G and H). No

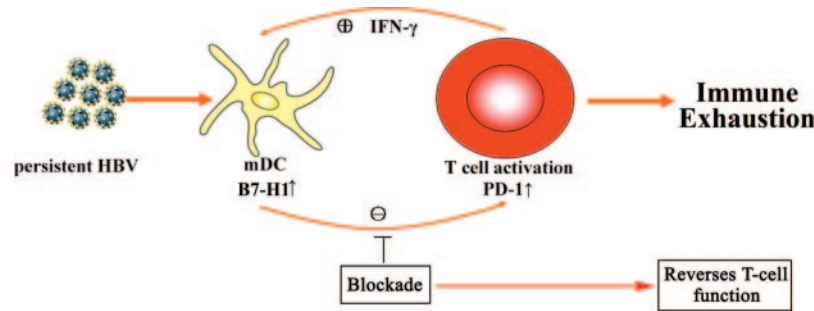


**FIGURE 5.** The blockade of B7-H1 increases the frequency of HBV-specific IFN- $\gamma$ -producing effector T cells and type 1 cytokine production. A, Purified T cells from patients were stimulated with HBcAg-pulsed autologous MoDCs in the presence or absence of anti-B7-H1 for 48 h. IFN- $\gamma$ -producing T cells were assayed using ELISPOT and are represented as IFN- $\gamma$  spot-forming units. Data shown are representative of eight independently performed experiments. B, Representative dot plots of IFN- $\gamma$  and IL-4 staining within T cell population. Values in the quadrant represent the percentage of the T cells that express IFN- $\gamma$  and IL-4. Data shown are representative of four independently performed experiments. C–F, Culture supernatants from ELISPOT assay with MoDCs and T cells at a ratio of 1:10 were analyzed for IFN- $\gamma$  (C), IL-2 (D), IL-12 (E), and IL-10 (F) using ELISA. Data shown are representative of eight independently performed experiments. Values of  $p$  are shown.

difference for the expression of some phenotypic markers including CD40, CD54, CD80, CD86, and HLA-DR on mDCs was found between IA patients and healthy controls; meanwhile, the addition of anti-B7-H1 did not alter the expression of these molecules on mDCs in MLR (data not shown). Thus, our results indicate that the blockade of B7-H1 modulates the cytokine production of mDCs, which may be responsible, at least in part, for enhancing T cell responses.

#### Blockade of mDC-associated-B7-H1 restores HBV-specific T cell responses

To demonstrate the effect of B7-H1 up-regulation of mDCs on HBV-specific T cell responses, T cells isolated from the CHB



**FIGURE 6.** Schematic figure of how the B7-H1/PD-1 pathway may mediate the exhaustion of virus-specific T cells in chronic HBV infection. HBV infection initially induces mDC-mediated T cell activation. However, viral persistence, indicated by high viral Ags, results in PD-1 up-regulation on T cells. Subsequently, activated T cells appear to produce an amount of inflammatory cytokines, such as IFN- $\gamma$ , which can lead to B7-H1 up-regulation on mDCs in vivo. Blockade of B7-H1 signal pathway can partly restore T cell function. Thus, these mDCs, probably serving as an important factor, mediate T cell immune exhaustion via the PD-1/B7-H1 pathway in CHB.

patients were stimulated with HBcAg-pulsed autologous MoDCs in the presence or absence of anti-B7-H1. The T cells were then analyzed by IFN- $\gamma$  ELISPOT and intracellular cytokine staining. We first detected the expression of B7-H1 and costimulatory molecules CD86 and CD80 on MoDCs. We found that the expression of B7-H1, CD86, and CD80 were all significantly up-regulated on mature MoDCs compared with immature MoDCs in CHB patients and HC subjects. Furthermore, CHB patients were found to exhibit a significantly higher B7-H1 expression, but lower CD86 and CD80 expression on mature MoDCs in comparison with HC individuals (data not shown).

More important, we found that blocking B7-H1 on mDCs could result in an increased number of IFN- $\gamma$ -producing HBV-specific T cells compared with a group treated with control Ig ( $p < 0.05$ ; Fig. 5A). This result was confirmed by similar data obtained by intracellular cytokine staining (Fig. 5B). We further detected the release of cytokines IL-2, IFN- $\gamma$ , IL-12, and IL-10 in the supernatants of the above culture system by ELISA. The findings revealed that the addition of anti-B7-H1 significantly increased the release of IL-2, IFN- $\gamma$ , and IL-12, but decreased IL-10 production, compared with isotype control Ig ( $p < 0.05$ ; Fig. 5, C–F). These findings indicate that B7-H1 up-regulation on mDCs of CHB patients may not only inhibit IL-12 production of MoDCs themselves, but also suppress autologous HBV-specific T cells to produce Th1-associated cytokines, whereas blockade B7-H1 signals on MoDCs can, at least in part, restore the functions of both MoDCs and HBV-specific effector T cells.

## Discussion

Recent studies have shown that functional defects in DCs may play an important role in the host-specific T cell immune tolerance and viral persistence during chronic HBV infection (17–19). However, the molecular mechanism of how the impaired mDCs in CHB patients influence HBV-specific T cell immune tolerance remains elusive. In this study, we demonstrate that chronic inflammation can induce B7-H1 up-regulation on mDCs, which may contribute to the defective HBV-specific T cell function. Importantly, the blockade of the B7-H1 signaling pathway can reverse, at least in part, the exhausted HBV-specific T cells.

In this study, we found that B7-H1 expression on circulating mDCs was up-regulated in CHB patients, as compared with healthy subjects. We conclude that this up-regulation of B7-H1 on mDCs may, partly, if not all, result from chronic inflammation. First, AIH patients enrolled in this study, who exhibited strong inflammatory liver disease in vivo that was not of viral

etiology, also displayed a significant B7-H1 up-regulation on mDCs. Second, in these CHB patients, the up-regulation of B7-H1 on mDCs seemed more significant in IA patients, who generally were characterized by higher ALT levels and lower viral load than that of the IT subjects. Third, the B7-H1 up-regulation in IA patients was positively correlated with serum ALT levels. This hypothesis was further supported by the finding that B7-H1 protein is strongly up-regulated by IFN- $\alpha$  and IFN- $\gamma$ , which was also in line with previous findings that proinflammatory cytokines, including IFN- $\gamma$  and TNF- $\alpha$ , could induce B7-H1 up-regulation on hepatocytes and endothelial cells (40, 41). These data also support the assumption that IT patients have a low endogenous immune response to HBV (37, 42) and, thus, have a lower expression of B7-H1 on mDCs compared with IA patients. Collectively, our data strongly suggested that chronic inflammation may contribute to B7-H1 up-regulation on mDCs in HBV-infected patients. However, we cannot exclude that virus may directly cause B7-H1 up-regulation on mDCs in CHB patients because of the detection of HBV DNA in peripheral mDCs (43).

Because mDCs play a pivotal role in Ag presentation and the regulation of effector T cells, functional deficiencies in the mDC-T cell interaction have been proposed to be responsible for HBV persistence (19). In this study, we provided evidence that the B7-H1-mediated coinhibitory signal may be involved in the defective T cell function. In accord with previous reports (18, 19), we also found that healthy CD4 and CD8 T cells were hyporesponsive to mDCs from CHB patients compared with allogeneic healthy mDCs, suggesting that an intrinsic defect of these mDCs may be responsible for defective stimulatory capacity. Especially, mDCs of CHB patients express decreased costimulatory CD80 and CD86 molecules, but increased inhibitory B7-H1 molecule. This striking alteration in the mDC repertoire may tip the balance between inhibitory and stimulatory signals delivered to T cells toward exhaustion, resulting in peripheral immune tolerance. The blockade of B7-H1 signaling by mDCs could reverse T cell proliferation defects and enhance the type 1 cytokine production. This recovery was closely associated with increased IL-12 and decreased IL-10 secretion in the coculture supernatant. Together with other recent studies of HIV-1 (30) and HBV (35) infection in humans, this report suggested that up-regulation of B7-H1 on mDCs may lead to increased inhibitory signals mediated by B7-H1 and its receptors, thus promoting T cell functional deficiency in chronic HBV infection and perhaps subsequently facilitating viral persistence. In this study, a significant, positive correlation between

B7-H1 expression on mDCs and plasma HBV DNA also supported this finding, although the cause-to-effect relationship between B7-H1 expression and viral replication needs future longitudinal investigation. In addition, whether the inhibitory signals probably delivered by B7-H1 on hepatocytes and liver-resident Kupffer cells may take on added significance on HBV-specific CD8 T cell exhaustion or immune tolerance is under investigation.

Moreover, we further sought to demonstrate the physiological role of B7-H1 up-regulation in the impaired HBV-specific T cell response. We observed that the blockade of MoDC-associated B7-H1 signals could enhance HBV-specific IFN- $\gamma$ -producing T cell frequency and type 1 cytokine production. This recovery following Ab treatment was also closely associated with increased IL-12 and decreased IL-10 production by mDCs. These data suggest that B7-H1 up-regulation on mDCs may be responsible for the defective HBV-specific T cell function in chronic HBV infection. In addition, Ag- or virus-pulsed autologous MoDCs have recently shown great clinical therapeutic potentials in HIV (11) and HBV (12, 44) infection. Thus, blockade of B7-H1 may improve the efficiency of MoDC-based therapeutic vaccine for CHB.

Overall, our study indicates that inflammatory cytokines may generate B7-H1 up-regulation on mDCs during chronic HBV infection, which may subsequently mediate virus-specific T cell exhaustion in chronic HBV-infected patients (Fig. 6). Blocking the B7-H1-mediated pathway can revitalize the exhausted virus-specific T cell repertoire. Thus, our findings begin to shed light on the understanding of immune pathogenesis during CHB infection and further support the notion that the blockade of the B7-H1-mediated inhibitory pathway may represent a potential therapeutic strategy against CHB.

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## Disclosures

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