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Impact of HBeAg on the maturation and function of dendritic cells

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ABSTRACT

Objectives: HBV infection typically leads to chronic hepatitis in newborns and some adults with weakened immune systems. The mechanisms by which virus escapes immunity remain undefined. Regulatory dendritic cells (DCregs) contributing to immune escape have been described. We wondered whether or not HBeAg as an immunomodulatory protein could induce DCreg which might subsequently result into HBV persistence.

Methods: The immunophenotyping, T-cell activation and cytokine production were analyzed in HBeAg-treated DCs from normal or cyclophosphamide (Cy)-induced immunocompromised mice.

Results: HBeAg tended to promote bone marrow derived DCs (BMDCs) from Cy-treated mice into $CD11b^{high}PIR\text{-}B^{+}$ regulatory DCs exhibiting the lowest T-cell stimulatory capacity and interleukin (IL)-12p70 production compared with controls. Neutralization of IL-10 significantly inhibited the regulatory effect of these DCs on T-cell stimulation of mature DCs. After lipopolysaccharides (LPS) stimulation, marked phosphorylation of Akt was detected in HBeAg treated DCs from immunocompromised mice. Blocking the PI3K-Akt pathway by LY294002 led to an enhancement of IL-12 production. PI3K signalling pathway appears to be involved in the decreased IL-12 secretion by HBeAg treated DCs.

Conclusions: These findings suggest that HBeAg may program the generation of a new DC subset with regulatory capacity under the condition of immunosuppression, which may presumably contribute to the persistent HBV infection.

1. Introduction

Chronic hepatitis B (CHB) is an ongoing worldwide health problem, affecting more than 350 million people globally.[1] The persistent infection of hepatic B virus (HBV) is a major cause of liver cirrhosis, hepatic failure and hepatocellular carcinoma. It is widely accepted that immune tolerance is a possible key factor for CHB, but its mechanisms remain elusive. One or more antigens of HBV may utilize diverse pathways to suppress the anti-HBV immune response and induce HBV-specific tolerance.

Hepatitis B e antigen (HBeAg), a secretory form of the nucleocapsid antigen, is translated from the preC transcript, sharing an overlapping reading frame with hepatitis B core antigen(HBcAg).[2] Although HBeAg is not necessary for infection or replication of HBV,[3] it is believed to be required for the establishment of chronic infection and probably responsible for the immunomodulation of host immune responses during CHB.[4,5] HBV establishes chronic hepatitis mainly by vertical transmission from HBeAg-positive mothers to neonates, as the immune system of neonates has not yet fully developed.[6] Significantly higher chronicity rate (up to 90%) is observed in this setting, whereas in HBV infected adolescents or adults, 5%-10% lead to a chronic carrier state.[7] Interestingly, babies infected perinatally with an HBeAg-negative mutant form of HBV experience an acute or fulminant acute course of infection rather than a persistent infection.[8] In addition, studies have found that immunosuppressed persons infected with HBV are more likely to develop chronicity.[9,10] AASLD guideline of CHB has categorized persons needing immunosuppressive therapy and HIV-infected individuals into high risk groups that should be tested for HBV infection and immunized if seronegative.[1] Thus, the tremendous difference in chronicity rates is supposed to be closely related to HBeAg and the immunologic

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status of the host at the time of infection. However, the function of HBeAg under different immune status has not been studied explicitly until now. There is a lack of well-established animal models of immunosuppression to study the pathogenesis of CHB.

As the most potent antigen presenting cells (APCs), dendritic cells (DCs) play a key role in the initiation and maintenance of specific T-cell immunity.[11–13] Studies have shown that defects in DC function are important factors in the host-specific T-cell immune tolerance to viral infection, rather than functional defects in T-or B-cells.[14,15] Additionally, DCs are not a single cell type, but rather a heterogeneous population of cells with high developmental plasticity. In recent years, considerable insight has been developed into a new subset of DCs with regulatory function named regulatory DCs (DCregs), and the role played by these DCregs in down-regulating immune responses and inhibiting inflammation has been shown in some in vivo models of autoimmune or inflammatory diseases.[16,17] We wondered whether DCs could acquire a tolerogenic or regulatory phenotype in response to specific stimuli of HBV, which in turn aided viral immune escape. So far, the potential effects of HBeAg on DCs have not been rigorously elucidated. We hypothesized that HBeAg, as a recognized tolerogen, may direct DCs to differentiate into DCreg contributing to the tolerance of CHB.

In the present study, we investigated the effects of HBeAg on bone marrow derived DCs (BMDCs) from mice in different immunologic status. The result showed that being incubated with HBeAg promoted DCs from immunocompromised mice to differentiate into a new regulatory DC subset that are phenotypically and functionally different from their classic conventional DCs. These findings provide further understanding of the impact of HBeAg on DCs and may ultimately benefit the development of new DC-based immunomodulatory strategies for chronic hepatitis B.

2. Methods

2.1. Mice and cyclophosphamide treatment

C57BL/6(B6) and BALB/c mice (6-8 weeks old, weighing 18-25 g) were purchased from Shanghai Slac Laboratory Animal Center, Chinese Academy of Sciences (Shanghai, China). Cyclophosphamide (Cy) was purchased from Jiangsu Hengrui Medicine Co. (Lianyungang, Jiangsu, China). The mice were all housed in specific pathogen-free facilities and maintained under constant temperature (20-22 °C), controlled humidity (45%-55%) and a 12-h light/dark cycle (lights on from 08:00 to 20:00). All experimental procedures were approved by the Institutional Animal Committee of Wenzhou Medical University and all mice received care throughout the experiment in accordance with "Guide for the Care and Use of Laboratory Animals".

The B6 mice were randomly allocated into 2 treatment groups in this study (n = 20 for each group). One group was administered intraperitoneally with a single dose of 200 mg/kg body weight (BW) of Cy 3 days before femurs and tibiae being removed and purified from the surrounding muscle tissues. Another group of healthy mice was kept as normal controls (normal group) and injected intraperitoneally with PBS similarly. The dosage and treatment regimens about Cy used here were based on the previous studies of immunosuppressed model.[18–20]

2.2. Generation of bone marrow-derived dendritic cells and cell cultures

BMDCs from the two groups were prepared according to established protocols with minor modification.[21,22] Briefly, cells were gathered from C57BL/6 bone marrow and cultured at a density of 2×10^6 cells/ml in 6-well plates in RPMI1640 (Gibico, USA), supplemented with 10% fetal calf serum (Gibico, USA), 1% penicillin/streptomycin, 1 ng/ml of recombinant mouse IL-4 and 10 ng/ml GM-CSF(PeproTec, London; United Kingdom) at 37 °C, 5%CO_2. On day 6, non-adherent and loosely adherent immature DCs(iDCs) were harvested and sorted by CD11c magnetic microbeads and a MiniMACS separator (Miltenyi Biotec, Germany) to obtain highly purified DCs. The cell viability after magnetic beads sorting was assayed with trypan blue staining. The percentage of CD11c positive cells was detected by flow cytometry.

HBeAg was purchased from Beijing Kewei clinical diagnostic reagents Co, Ltd., Beijing, China. To investigate the influence of HBeAg on DC, 5 μg/ml HBeAg was added into the CD11c+ cells for 24 h. In some instance, the cultures were supplemented with 100ng/ml lipopolysaccharide (LPS, Sigma -Aldrich;USA) for another 24 h or 100 nM PI3K inhibitor LY294002 (Beyotime Institute of Biotechnology, Haimen; China) for 1 hour in advance, then followed with LPS stimulation.

2.3. Flow cytometry

To characterize and compare the phenotype of DC populations, flow cytometry was performed. DCs were harvested and washed. The cells were then incubated in cold buffer and subsequently stained for 30 min with the following APC-, PE-, FITC-labeled monoclonal antibodies or appropriate isotype controls: CD11b, Ia(BioLegend, USA), PIR, CD86(eBioscience, San Diego, USA). Stained cells were analysed in an Elite flow cytometer (Coulter, Hialeah, FL).

2.4. Real-time quantitative RT–PCR analysis of PIR-A and PIR-B

RNA was extracted from DCs by Trizol reagent (Invitrogen, Carlsbad, CA, USA) and the cDNA synthesis of RT–PCR was performed using a commercial kit (Toyobo, Osaka, Japan) according to the manufacturer's instructions. Then, the PCR reaction mixture was prepared by SYBR Green Real-time PCR Master Mix-Plus (Toyobo, Osaka, Japan). Amplification of cDNA was performed with a common forward primer (5′-CCTGTGGAGCTCACAGTCTCAG-3′) and the PIR-A-specific primer (5′-CCCAGAGTGTAGAACATTGAAGATG-3′) or PIR-B-specific primer (5′-GTGTTCAGTTGTTCCCTTGACATGA-3′). Amplification of β-actin transcription with the primers 5′-CTG GCA CCA CAC CTC CTA CA-3′ and 5′-AGT ACT TGC GCA CAG GAG GA-3′ was performed as a control.

2.5. Allogeneic-mixed lymphocyte reactions (MLRs)

T-cells were isolated and purified from healthy BALB/c spleen by nylon wool columns and CD4+ T isolation kit (Miltenyi Biotec, Germany), according to the manufacturer's instructions. Primary MLRs were performed as previously described. Briefly, graded concentrations of DCs from different culture conditions were co-cultured in U-bottom 96-well plates with constant number of allogeneic T-cells (1×10^5cells/200 μl) at different stimulator/responder(DC/T-cell) rations(1:5, 1:10, 1:20) for 96 h. The T-cell stimulatory activity of DC populations in MLR was expressed as stimulation index (SI) value and measured using CCK-8 cell proliferation array kit (Beyotime Institute of Biotechnology, Haimen, China), in accordance with the manufacturer's instruction. SI values of the primary MLR consisting of naïve 10^5 BALB/c CD4+ T-cells, 10^4 mature DCs (mDCs) and indicated numbers of HBeAg-treated DCs were detected to test the regulatory function. In neutralizing cultures, anti-IL-10 antibodies (R&D Systems, Minneapolis, MN) were used at the beginning of the MLR.

2.6. Cytokine production

The concentration of IL-12p70 and IL-10 in culture supernatants of pure DCs under different treatments was estimated by

ELISA using corresponding enzyme-linked immunosorbent assay kits (R&D Systems, Minneapolis, MN).

2.7. Western blotting

Expression of p-Akt and Akt were determined by western blot according to standard protocols. The rabbit anti-Akt (Abcam, UK, 1:1000), rabbit anti-p-Akt (Cell Signalling, USA, 1:1000) and a horse peroxidase (HRP) -conjugated secondary goat anti-rabbit antibody (Biosharp, China, 1:5000) were used. Visualization was achieved by chemiluminescence (ECL).

2.8. Statistical Analysis

All data were reported as mean ± standard deviation (S.D.). Statistical analysis was performed by Student *t* test or one-way ANOVA followed by post-hoc tests (using LSD-t or Dunnett's T3) for multi group comparisons with SPSS18.0 program. All P values < 0.05 were considered significant.

3. Results

3.1. HBeAg educates DCs to differentiate into a phenotypically distinct population

The percentage of viable DCs detected with trypan blue staining was not significantly changed after purification compared with before in both two mice groups [(96.61 ± 0.34) % vs (96.08 ± 0.22) %, P > 0.05; (96.28 ± 0.23) % vs (96.15 ± 0.25)%, P > 0.05]. More than 95% cells were positive for CD11c as assessed by flow cytometry (Figure 1A).

To clarify the role of HBeAg in regulation of the phenotype of DCs in the normal mice and Cy-induced immunocompromised mice, we applied exogenous HBeAg(5 μg/ml) addition in DCs for 24 h. There were dramatic enhancement in the expression of CD11b and paired immunoglobulin-like receptor-A/B (PIR-A/B) on DCs from both normal and immunocompromised mice (Fig. 1B). And Cy plus HBeAg treated group showed a higher expression level of CD11b and PIR than HBeAg treated DCs from normal mice. Besides, its proportion of CD11B+PIR+ DCs(75.9% ± 5.8%) was also significantly higher than HBeAg group(63.3% ± 4.1%)($P < 0.05$)(Fig. 1C). DC's surface molecules, including Ia and the costimulatory molecules CD86, all of which will be greatly up-regulated during the DC maturation. However, HBeAg was unable to change the expression of Ia and CD86. Furthermore, the unique phenotype of high expression of CD11b and PIR on HBeAg incubated DCs from Cy-treated mice remained unchanged even stimulated with LPS(100ng/ml), thus indicating that such DCs exhibit a relatively stable phenotype (Fig. 1B).

PIR-A and PIR-B are members of immunoglobin superfamily. We investigated that there was an up-regulation of PIR positive rate in DCs cultured with HBeAg for the first time. PIR-B is known as an inhibitory receptor, which consists of the activating-type PIR-A. Then we examined the mRNA of PIR-A and PIR-B respectively to detect which type dominantly expressed on cells by real-time quantitative PCR. The PIR-B mRNA in both two HBeAg treated groups increased significantly ($P < 0.05$), compared with that of the control groups. And the HBeAg plus Cy treated group showed a higher level of PIR-B mRNA than that of HBeAg-treated DC from

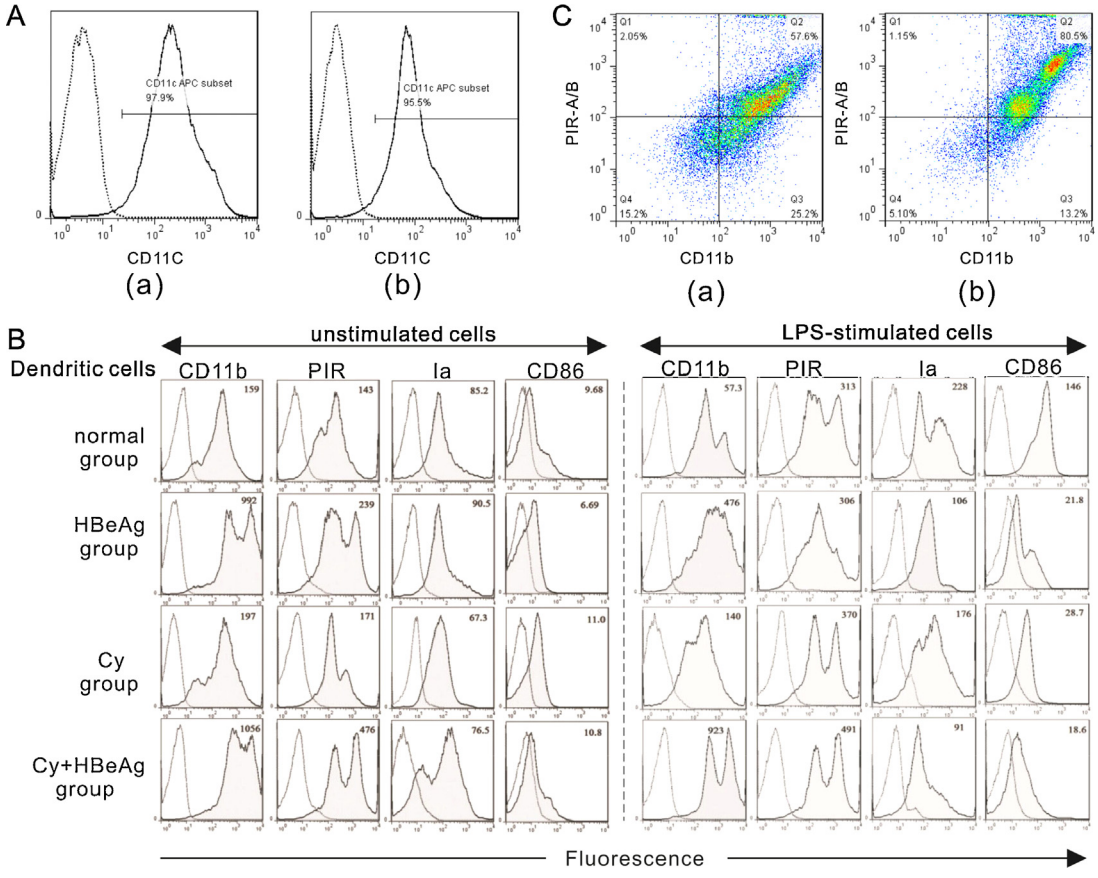

Figure 1. HBeAg drives differentiation of DCs into CD11b^{high}PIR^{high} DCregs in vitro. (A)Percentages of CD11c positive cells after purification (a, normal group; b, Cy-treated group). (B) DCs from normal or Cy-treated mice were stained with different mAbs and analyzed by flow cytometry in the presence or absence of HBeAg. With or without HBeAg, DCs were treated with LPS(100ng/ml) for 24 hrs. Then, the cells were harvested and analyzed by flow cytometry. (Dotted line, isotype control; solid line, specific mAbs; numbers in histograms indicate the gometric mean flurescence). (C) Percentages of CD11b^{+}PIR^{+} cell in HBeAg-treated DCs from normal (a) or Cy-treated mice (b).

normal mice (Fig. 2A). The expression of PIR-A mRNA showed no significantly difference among groups. LPS can induce higher PIR-A expression and lower PIR-B expression. So we detected the changes of PIR-A and PIR-B expression after the simulation of LPS (Fig. 2B.C). The result showed HBeAg plus Cy-treated DCs remained high expression of PIR-B without obvious change of PIR-A. However, the PIR-A mRNA significantly ascended after the administration of LPS in the groups without HBeAg.

3.2. HBeAg treated DCs show reduced T-cell stimulatory capacity and partially IL-10-dependent regulatory function

To further determine the functional properties of DCs, we analyzed their T-cell stimulatory capacity in mixed lymphocyte reaction (MLR) experiments. As expected, the T-cell stimulatory capacity of immature DC (iDC) from both normal and Cy-treated mice was very low in comparison to that observed for mature DC (mDC). More importantly, the results showed that DCs from both the two groups incubated with HBeAg tended to have a considerably diminished T-cell stimulatory activity ($P < 0.05$) as compared with mDC (Fig. 3A). And Cy + HBeAg group showed a lower T-cell stimulatory capacity than HBeAg-treated DCs($P < 0.05$) at a DC:T ratio of 1:5 and 1:10. Our results showed that HBeAg was not an efficient inducer for DCs to generate T-cell proliferation (Fig. 3A). In addition, to determine if these HBeAg-treated DCs had regulatory capacity, we tested their effect on the T-cell activation of mDCs.10^3 HBeAg-treated DCs from immuno-compromised mice could suppress the MLR induced by 10^4 mDC by almost 35%, and when mixed at a 1:1 ration, the degree of suppression increased to approximately 50%(Fig. 3B). IL-10, secreted by many kinds of cells with regulatory functions, can induce T-cell unresponsiveness when present during T-cell activation.[19] Given that regulatory function of bone marrow derived DCregs has been attributed to IL-10,[23] we wondered that whether IL-10 contributed to the regulatory function of these HBeAg treated DCs. Results showed that blockade of IL-10 by anti-IL-10 antibody reduced the regulatory capacity of these cells by approximately 45%($P < 0.05$), indicating a partially IL-10-dependent regulatory function(Fig. 3C).

3.3. Cytokines production by DC under different culture condition

It appears that IL-12 and IL-10 are both important cytokines during the immune response to infection, which play antagonist roles. Spontaneous IL-12 and IL-10 production of DCs is shown in Fig. 4A. HBeAg induced statistically significantly higher level of IL-12 and IL-10 secretion than the controls. This study gave a result of zero of IL-12p70 secretion of untreated iDCs, which was consistent with the previous report.[24]

It has been reported that LPS stimulation induced significant up-regulation of cytokines including IL-12 and IL-10. As expected, exposure of iDCs to LPS alone showed dramatic up-regulation in the production of these two kinds of cytokines. However, when HBeAg-treated DCs were stimulated with LPS, the increase secretion of IL-12 was impaired (Fig. 4B). Maturation in the presence of HBeAg may prevent the up-regulation of IL-12 secretion with the stimulation of LPS. As the expression of cytokines is known to be up-regulated by LPS in cDCs, our observations may indicate that HBeAg-treated DCs showed a unique cytokine profile.

3.4. PI3K-Akt plays a crucial role in HBeAg-mediated IL-12 inhibition

Previous studies have reported that phosphoinositide 3-kinase (PI3K) exerts negative regulation on IL-12 transcription.[24,25] To investigate this signaling mechanism involved, we detected the

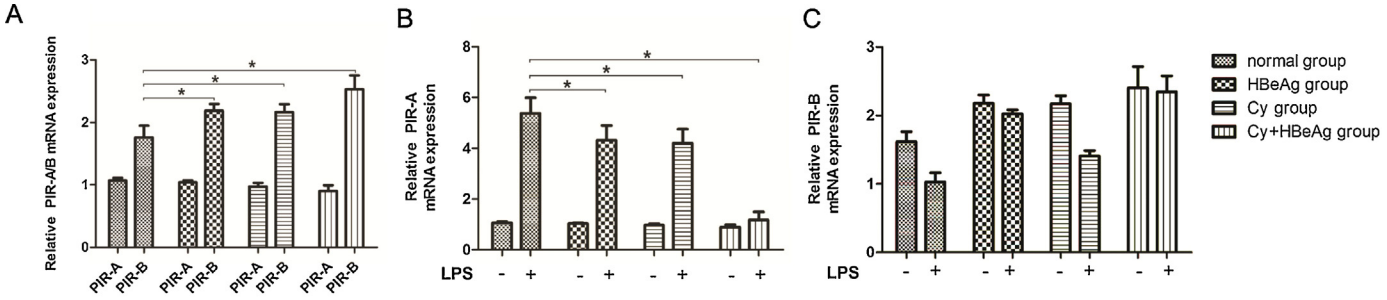

Figure 2. Effects of HBeAg on the expressions of PIR-A and PIR-B mRNA. Results are normalized to β-actin. Each bar represents mean ± S.D. (A) Expression levels of PIR-A and PIR-B transcripts in DCs from normal or Cy-treated mice in the presence or absence of HBeAg. (B.C) Expression levels of PIR-A or PIR-B in DCs from different groups stimulated with LPS (100ng/ml).

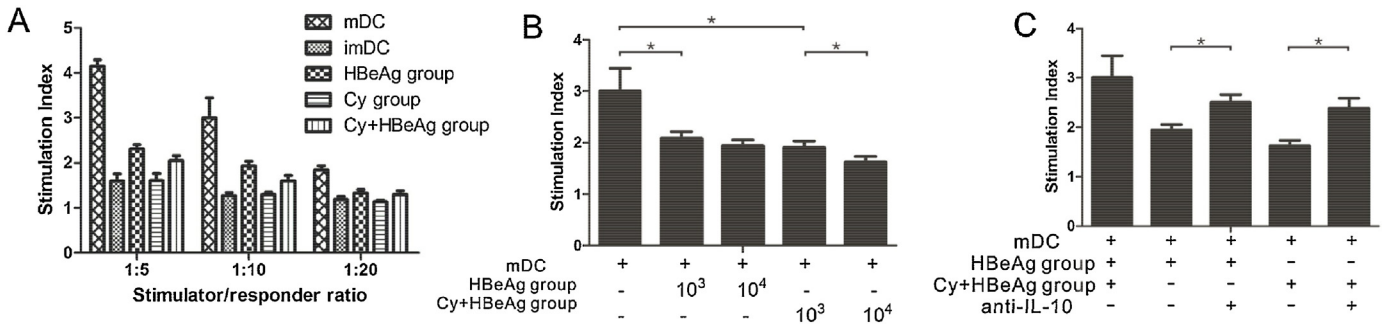

Figure 3. Low T cell proliferation-inducing ability of DCs in the presence of HBeAg. (A) DCs function was analysed in a MLR by incubating DC obtained under different culture conditions with allogenic T lymphocytes from BALB/c mice at the indicated ratios. T-cell proliferation induced by iDCs, mDCs or DCs in the presence of HBeAg was measured. Mean ± S.D. of stimulation index of 5 separate experiments are shown. (B) Inhibition of MLR stimulated by 10^4mDCs by indicated numbers of HBeAg-treated DCs. (C) Partial reversal of HBeAg-treated DCs function by anti-IL-10 antibodies.

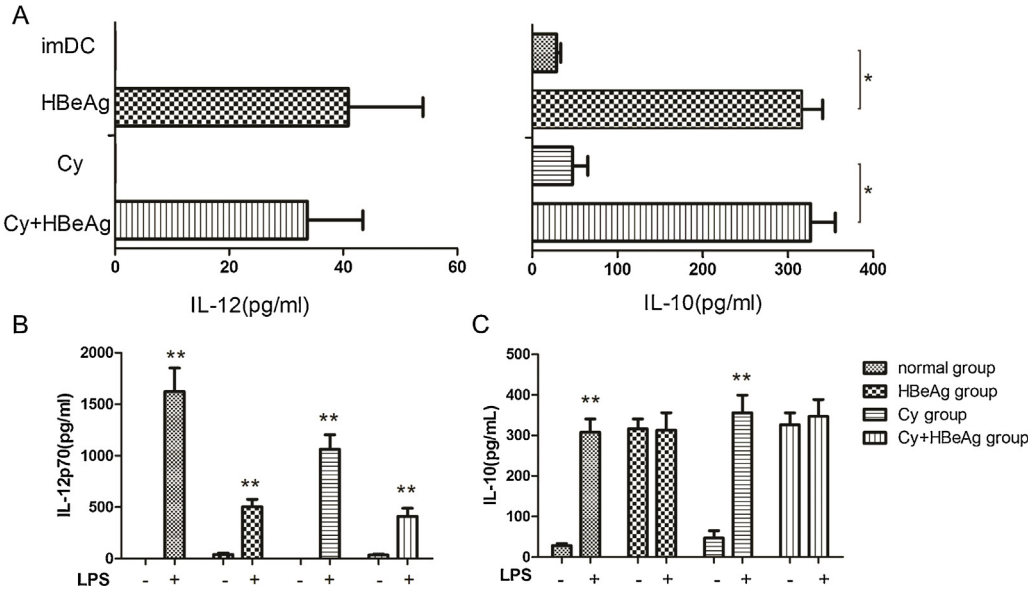

Figure 4. Effects of HBeAg on the production of IL-12 and IL-10. (A) The cell-free supernatants of different cultures were collected and analyzed for IL-12p70 and IL-10. HBeAg induced IL-12p70 and IL-10 production in DCs. (B.C) The expression of IL-12p70 and IL-10 by DC was assessed by ELISA after the stimulation of LPS for up to 24 h. Each column represents the mean ± S.D. of four independent experiments. Statistical significance was calculated by t-test (**$P< 0.01$ versus each corresponding group without LPS).

expression level of phosphorylated Akt (pAkt) by western blot and found that the expression level of pAkt was up-regulated within 30 minutes in DCs treated with HBeAg, especially in DCs from Cy group (Fig. 5A). Then we employed the antagonists of PI3K signaling to the cultures. Pretreatment of PI3K inhibitor (LY294002) could augment LPS-induced IL-12 production, whereas the levels of IL-10 in four groups were not strikingly altered in LY294002-pretreated DCs (Fig. 5B.C).

4. Discussion

Recent studies have shown some new mechanisms of HBV escaping immune responses. Lang et al.[26] found that HBeAg may contribute to the pathogenesis of CHB infection via interacting and co-localizing with TLR proteins, thus suppressing TIR-mediated activation of both NF-κB and IFN-β. Purvina et al.[27] demonstrated that HBeAg inhibited T lymphocyte proliferation through the IL-1 signalling, which in turn aids the establishment of chronic infection. Another study reported that HBeAg could modulate immune responses by down-regulating IL-18 mediated expression of IFN-γ which had direct antiviral effects against HBV.[28] A recent paper has reported that HBeAg had a negative effect on the generation of DCs.[29] A high incidence of CHB in infants with immature immune system or adults with lower immunity has been reported. However, all the works mentioned above did not take a look at the role of HBeAg on DCs from immunocompromised immune status. Because the subset of regulatory DCs were identified and considered to be important in the induction and maintenance of immune tolerance or down-regulation of immune response,[23,30,31] we conducted experiments to elucidate whether HBeAg can play an active role in directing the differentiation of DCs with regulatory function propagated from bone marrow precursors of normal or immunocompromised mice. Cy is a well-described immunosuppressant which has previously been used to induce an immunosuppressed mouse model to mimic the infected persons with low immunity[18,20] and this immunosuppressed model has been the most practical one so far. So, this Cy-induced immunosuppression model was chosen to examine the effect of HBeAg on DCs in weakened immune systems.

In our study, we first demonstrated that HBeAg could induce bone marrow-derived progenitors to differentiate into a phenotypically distinct DC population with high expression of CD11b and PIR-B from both normal and Cy-treated mice. And we observed a higher expression level of these two kinds of cellular surface protein on the latter according to the results from flow cytometric analysis and RT-PCR experiments. In addition, Cy administration also had great influence on the function changes of DCs incubated with HBeAg. These data may be useful for future investigation of the pathogenesis of CHB in the setting of immunocompromised status, realistically mimicking clinical scenarios.

CD11b, which is highly expressed on DCregs described by Cao XT et al.,[32] may not be a specific marker for DCreg, because high CD11b expression is also observed in other cell types like microglial cells, macrophages and MDSCs. However, it is a relatively stable cell molecule on the DCreg membrane according to the previous studies.[32] PIR-A/B, responsible for both specific and nonspecific immune responses,[33] is believed to play an important role in the function regulation of DCs. PIR-B is expressed on B lymphocytes, myeloid cells and dendritic cells, and the role played by PIR-B in the negative regulation of immune response has been documented.[34,35] The orthologous of PIR-B in humans are immunoglobulin-like transcript (ILT)3 and ILT4, which are critical for DCs to acquire tolerance.[36] To our knowledge, there is no report about the expression of PIR-A/B on DCs treated with HBeAg. Our study found that the inhibitory member PIR-B necessary for maintaining the steady condition of DCs appeared to be dominant compared with PIR-A, which was consistent with the previous research reported by Zou Ping and Pereira et al.[37,38] The PIR-B expression of HBeAg treated DCs was remarkably up-regulated, but no significant difference in PIR-A was observed among the groups. Thus, it was speculated that HBeAg might affect the function of DC by up-regulation of PIR-B rather than PIR-A, thus providing a novel molecular mechanism for DCs to acquire tolerance. Further experiments showed that LPS could not reduce the high expression of CD11b and PIR-B, indicating that HBeAg-induced DCs had a relatively stable phenotype. Therefore, we propose another possible new subset of DCreg with a distinct phenotype, which may be involved in the induction of tolerance of HBV.

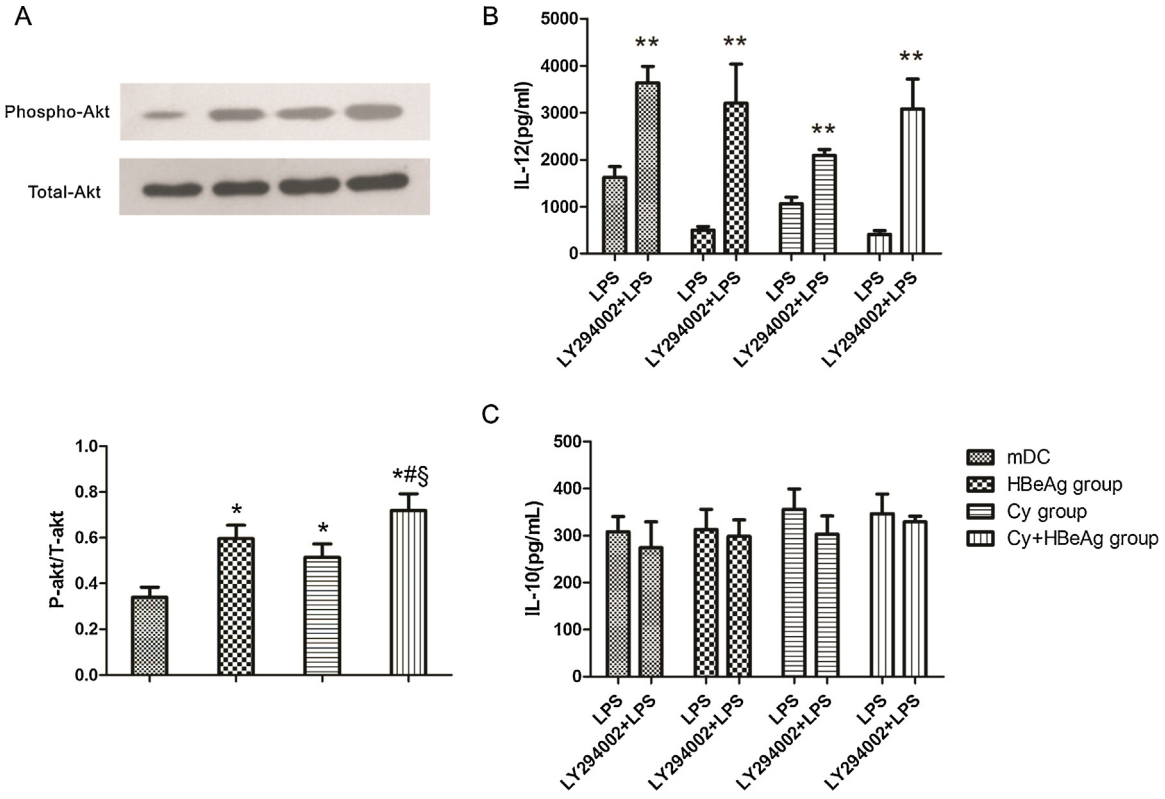

Figure 5. PI3K signal activation was crucial for HBeAg-mediated suppression of IL-12. (A) Whole-cell lysates from DCs in different culture conditions with LPS (100ng/ml) treatment for 30 minutes were subjected to Western blot analysis of phospho-Akt. Results are normalized to total Akt. Each column represents the mean ± S.D. of three independent experiments.*, # and § indicate higher expression of phospho-Akt than LPS, HBeAg+LPS and Cy +LPS groups, respectively and *P*-value significance ($P < 0.05$) was calculated by ANOVA. (B.C) LY294002 was used or not to treat DCs in different groups for 1 hour before the further stimulation of LPS (100ng/ml). And the production of IL-12p70 and IL-10 in the cell-free supernatants was assayed by ELISA.

Apart from the distinctive phenotype, another feature of DCs is their ability to promote T-cell proliferation. Thus, MLR was conducted to determine this capacity of HBeAg-treated DCs. As there is no effective and appropriate method to isolate both CD11b and PIR-B positive DCs, total HBeAg-treated DCs were detected. A clearly reduced T-cell proliferation compared to mDCs was observed in HBeAg incubated DCs. When DC/T was 1:5 or 1:10, DCs treated with HBeAg from immunocompromised mice tended to have the lower T-cell stimulatory capacity as compared with HBeAg treated cells from normal mice, suggesting that Cy might promote the attenuation of DCs' T-cell stimulatory activity caused by HBeAg. But when DC/T was 1:20, there was no significant difference between these two HBeAg-treated groups, perhaps because the quantity of DC was not enough to promote T-cell proliferation. Furthermore, HBeAg treated DCs from immunocompromised mice are more potent inhibitors of MLR with a partially IL-10-dependent regulatory function.

The inflammatory factors are believed to be involved in the pathogenesis of CHB. HBeAg can affect key cytokine production during the establishment of CHB.[28] IL-12 is produced by DCs early in immune response and exerts its biological function by driving Th1 cell activation and differentiation, and the autocrine IL-10 is involved in a regulatory mechanism in suppressing IL-12 production in DCs.[39] Here, we observed that DCs in groups with HBeAg spontaneously released relatively a low level of IL 12p70 and a high level of IL-10, which may be related to the potency in inhibiting a primary MLR. And another outcome from this study was that after the stimulation of LPS, the IL-12 production of HBeAg-treated DC increased, but not to the same magnitude. However, the up-regulation of IL-10 was not impaired. Therefore, it is not likely that IL-10 was the decisive factor in the suppression of IL-12 in HBeAg treated DCs upon stimulation with LPS.PI3K-Akt signal pathway has been reported to play a central role in the response of DC to environmental stimulus, including the regulation of IL-12 secretion.[24,25] So, in order to identify the possible mechanisms for the impacts of HBeAg on the cytokine production of DCs, the phosphorylation level of phosphor-Akt was measured by western blot. We observed an enhanced expression level of phosphor-Akt in HBeAg-treated DCs, especially in the Cy group with the highest relative protein expression level. Pretreatment of DCs with LY294002, the specific inhibitor of the PI3K pathway, could significantly augment LPS-induced IL-12 production. These data suggest that the PI3K pathway is likely to be involved or at least played a partial role in the HBeAg inducing down-regulation of IL-12 production of DCs.

In conclusion, we found that HBeAg could program bone marrow progenitors into a DC subset in vitro with considerably up-regulated expression of CD11b and PIR-B, a decreased T-cell stimulatory capacity and a suppressed production of IL-12. It is worth mentioning that these phenomena were more evident in DCs generated from Cy-treated immunocompromised mice. PI3K signalling activation was related to the down-regulation of IL-12 secretion. Our findings provide novel information for the current recognition of the formation of persistent HBV infection and may cause some meaningful implications. Our experiments did not investigate the underlying molecular mechanisms contributing to the generation of such regulatory DCs, and we need further in vivo experiments designed and tested to evaluate the immunosuppressive moderation function of HBeAg and validate the existence of regulatory DCs in HBV transgenic mice or patients with chronic HBV infection.

Conflict of interest: The authors declare no conflict of interest.

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