Phenotype and function of monocyte-derived dendritic cells in neonates born to Hepatitis B virus-positive mothers

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Keywords: dendritic cells, hepatitis B, intrauterine transmission, neonates, phenotypic molecules

Abbreviations

HBV	Hepatitis B Virus
DC	Dendritic cell
APC	antigen-presenting cell
MoDC	Monocyte-derived Dendritic cell
FCM	Flow Cytometry
MLR	mixed lymphocyte reaction
ELISA	enzyme-linked immunosorbent assay
pDC	plasmocytoid dendritic cells
CB	cord blood
CBMCs	cord blood mononuclear cells
HBeAg	hepatitis B e antigen
CLIA	chemiluminescence immunoassay
FQ-PCR	fluorescence quantitative polymerase chain reaction
GM-CSF	granulocyte-macrophage colony stimulating factor
IL-4	interleukin-4
TNF-α	tumor necrosis factor-α
SD	standard deviation

Summary

Hepatitis B Virus (HBV) infection in infancy or early childhood leads to high rate of persistent infection (25-90%). The immunological basis of high rate of viral persistence in vertically acquired HBV infections is not completely understood. Dendritic cells (DCs) are one of the most potent antigen-presenting cells (APC) and play pivotal roles in the enhancement or regulation of antiviral immune reactions. Aim of the present study was to investigate whether an HBV-infected maternal environment might influence the infants' DC phenotype and function. Monocyte-derived DC (MoDC) of neonates born to HBsAg-positive mothers were studied phenotypically by Flow Cytometry (FCM) and functionally by mixed lymphocyte reaction (MLR) and enzyme-linked immunosorbent assay (ELISA). An electron microscope was used to analyze the morphological changes of MoDC. MoDC from neonates whose maternal HBV DNA was>5×10^7 copies/ml showed a reduced surface expression of CD80, CD86, and HLA-DR as compared to that in neonates whose maternal HBV DNA was negative (CD80: t=3.238, P=0.002; CD86: t=3.543, P=0.001; HLA-DR: t=2.785, P=0.008). T-cell proliferation assays also showed an impaired allostimulatory capacity in comparison to that in neonates whose maternal HBV DNA was negative, especially in the cultures at a DC: T cell ratios of 1:5 and 1:10 (t=-5.442, P<0.001; t=-2.195, P=0.042). Therefore, it can be speculated that the presence of high level of HBVDNA in the maternal environment might lead to minor phenotypic and functional alterations of MoDC from neonates and subsequent deficits in T-lymphocyte activation may

contribute to viral persistence.

Keywords: dendritic cells, hepatitis B, intrauterine transmission, neonates, phenotypic molecules

Introduction

Hepatitis B virus (HBV) infection is a global pandemic with more than 257 million people suffering from chronic HBV infection worldwide, who are at high risk of developing end-stage cirrhosis and hepatocellular carcinoma [1]. In the most highly epidemic areas, especially mainland China, mother-fetus transmission of HBV plays an important role in the high prevalence of carrier status [2,3]. As we know, the key determinant of chronic infection is the age of infection. Chronic infection occurs in 90% of infected neonates and infants but in less than 5% of patients who acquire infection in adulthood [4]. The increased incidence of chronicity is attributed to the immaturity of the neonatal immune system and, especially, to the functional impairment of T cells [3,5]. Dendritic cells (DCs) are uniquely well-equipped in antigen-presenting function and act as key players in initiating T-lymphocyte activation against viral agents. The importance of DCs has been demonstrated by experiments showing that neonatal T cells can reach adult-like responses when stimulated with isolated allogeneic adult DCs [5]. The previous reports showed that the presence of HBV or its products in the maternal environment may alter the development of the DC systems of those newborns [6]. GUO *et al.* found that neonatal plasmocytoid dendritic cells (pDC) frequencies decreased when maternal HBV DNA loads are $>5\times10^7$ copies/ml [7]. Similarly, in utero exposure to HIV-1 has been shown to induce quantitative and qualitative changes in neonatal DCs [5,6]. Recent reports on the role of DCs in HBV infection have focused on adult life, while the phenotype and function of DCs in neonates born to HBsAg-positive

mothers has not been determined. The aim of the present study was to assess the phenotypic and functional consequences of the monocyte-derived DC (MoDC) in infants born to HBsAg-positive mothers with different HBV serological profiles. Considering the isolation of DC from cord blood (CB) is hampered by the very low numbers and immature differentiation stage of circulating DC precursors, the present study employed a good protocol to generate DC in vitro from CB mononuclear cells (CBMCs) by culture with GM-CSF, IL-4 and TNF-α to study phenotype and function of MoDC from Human CB [7-9].

Materials and methods

Study Subjects

All subjects included in this study were born to HBsAg-positive, HCV- and HIV-negative mothers who had no clinical or laboratory signs of active hepatitis B at the time of delivery. All neonates were born at term (between37 and 42 gestation weeks), and birth weight was greater than 2,500 g. According to the national recommendations, all neonates received one dose of 200 IU of hepatitis B immune globulin (HBIG) and their first hepatitis B vaccine (NCPC GeneTech Biotechnology Co. Ltd, China) within 24 h after birth. Hepatitis B vaccination was completed by another two doses of vaccine at the ages of 1 and 6 months.

At the time of delivery, 40 ml of cord blood was collected in a subset by using sterile

heparinized syringes. Peripheral blood samples from the mothers were obtained before delivery and femoral venous blood samples from all neonates were obtained at birth (before the administration of passive-active immunoprophylaxis) in EDTA tubes. Non-anticoagulant peripheral blood samples from neonates and mothers were also collected.

The research protocol was approved by the Human Investigation Committee at the Shanxi Medical University. Informed written consent was obtained from all mothers.

Serological HBV markers and HBV DNA

HBsAg and hepatitis B e antigen (HBeAg) were measured using chemiluminescence immunoassay (CLIA) kits (Roche Co. Ltd, Switzerland) for all mothers during pregnancy, and their neonates on the day of delivery before the administration of passive-active immunoprophylaxis. HBV DNA levels of mothers and neonates were determined by fluorescence quantitative polymerase chain reaction (FQ-PCR) assay (Da'an Gene Co.Ltd, Sun Yat-Sen University, Guangdong, China). HBV DNA loads > 1×10^3 copies/ml were defined as positive. HBV intrauterine transmission was defined as finding HBsAg and/or HBV DNA positive in the peripheral blood of neonates within 24 h after birth, before active or passive immunoprophylaxis [10,11].

Generation of MoDC from CBMCs

MoDCs were prepared from CBMCs mainly according to previously established

protocols [8,9]. CBMCs were isolated from freshly drawn heparinized cord blood by Ficoll-Hypaque density gradient centrifugation, washed two times, and resuspended at 2 × 10^6/ml in RPMI 1640 (RPMI 1640 medium was purchased from Boster Biological Technology co. Ltd, Wuhan, China).

After 3 h incubation at 37◦C in 5% CO_2 in 6-well plates, supernatant was discarded and adherent cells were incubated in RPMI 1640 plus 10% fetal bovine serum overnight. The nonadherent cells were gently removed and adherent cells were cultured in RPMI 1640 with 10% fetal bovine serum supplemented with 100 ng/ml of granulocyte-macrophage colony stimulating factor (GM-CSF) (Peprotech, USA) and 50 ng/ml of interleukin-4 (IL-4) (Peprotech, USA). Half of the medium was refreshed and cytokine was added at the middle concentration every 2 days. On the 5th day of incubation, 25 ng/ml of tumor necrosis factor-α (TNF-α) (Peprotech, USA) was added to the medium, and then the cells were collected on the 7th day.

Morphology of Dendritic Cells. The morphology of dendritic cells was observed by light microscope and transmission electron microscope.

Flow Cytometry of Surface Markers.

MoDCs were harvested on the seventh day and the expression of surface markers was analysed by flow cytometry (FCM) using conjugated monoclonal mouse-anti-human

antibodies: PE-anti-CD80, FITC-anti-CD86, and APC-anti-HLA-DR (all purchased from BioLegend, USA). Analysis was performed on a FACScan (Becton Dickinson) utilizing the CellQuest software. Quadrants were set according to staining with the respective isotype controls.

T Cell Stimulation

Mixed lymphocyte reaction (MLR) was performed to evaluate the allostimulatory activity of MoDCs with allogeneic responder cells from nonadherent CBMCs of healthy neonates. After being treated with 50 μg/ml of mitomycin at 37◦C for 45 min, MoDCs were plated at concentrations of 2×10^4, 1×10^4, and 5×10^3 cells per well separately, then incubated with allogeneic T lymphocyte at concentrations of 1×10^5 cells per well in triplicate. The total volume was adjusted to 200 μL/well, and then incubated for additional 96 h at 37◦C in 5% CO_2. Before the end of the culture, 20 μL/well tetrazolium salt (CCK-8) (purchased from Tongren, Japan) was added to the medium for 4 h. Meanwhile, T lymphocyte group (without dendritic cells incubated together) and only RPMI 1640 medium group were established as negative control group and background group respectively. Absorbance (A) was measured by ELX800G (Biotech, USA) at a detection wavelength of 450 nm and a reference wavelength of 630 nm. The results were expressed as stimulation index (SI) calculated by the following formula: stimulation index = (values of the sample – the background values)/ (values of the negative control – the background values).

Cytokine Secretion by MoDC

Supernatants from MLR culture were collected on the 4th day for detection of IL-10, IL-12and IFN-γ, using ELISA, which was purchased from R&D Systems (Minneapolis, MN, USA) and according to the manufacturer's instruction.

Statistical analysis

All data were expressed as mean ± standard deviation (SD) and were analyzed using SPSS v.22.0 software (SPSS Inc., Chicago, IL, USA). The Student's t test was used to determine the significant differences in mean values between two groups. Comparison of many groups was analyzed by a one-way ANOVA test (Student-Newman-Keuls method). Correlation analysis was performed by χ^2 test. $P<0.05$ was considered to indicate statistical significance.

Results

Population description

The study population included 48 neonates born to HBsAg-positive mothers, 6 neonates of whom were detected with serum HBsAg or HBV DNA positive, the incidence of HBV intrauterine transmission was 12.50% (6/48). 20 HBeAg-positive and 1 HBVDNA-positive neonates were found in these neonates. The percentage of HBeAg and HBV DNA positive in 48 mothers were 60.42% (29/48) and 66.67% (32/48), respectively.

Morphological Characteristics of MoDC by inverted phase contrast microscope

On the third day, the number of proliferative DCs increased and the size of some cells increased. With the extension of incubation time, more and more cells were induced, began to suspend and grew branched projections. On the seventh day, the DCs extended large and displayed a typical morphology with many fine dendrites.

Morphological Characteristics of MoDC by transmission electron microscope

After 7 days induction with recombinant GM-CSF, IL-4 and TNF-α, **MoDC** derived from neonates whose maternal HBV DNA was>5×10^7 copies/ml were irregular in form, and abundant in extended beard-like prick, mitochondria and rough endoplasmic reticulum in cytoplasm under transmission electron microscope (Fig.1).

HBV intrauterine transmission and Phenotype of MoDC and Cytokine secretion

After 7 days of culture, MoDC derived from HBV intrauterine transmission group showed a lower expression of the surface markers CD80, CD86, and HLA-DR compared to the HBV intrauterine non-transmission group. There were no statistically significant differences between two groups (CD80: t=1.483, P=0.145; CD86: t=0.752, P=0.456; HLA-DR: t=1.735, P=0.089) (Table1, Fig.2, Fig.3A). The results of cytokine measurements by ELISA analysis were shown in Table 1. The concentrations of IL-10, IL-12 and IFN-γ in MLR supernatants were comparable in both groups (IL-10: t=1.536,

P=0.131; IL-12: t=1.259, P=0.214; IFN-γ: t=1.154, P=0.254) (Table1, Fig. 3B).

Relationship between maternal HBeAg positivity and neonatal HBeAg positivity

Correlation analysis showed that neonatal HBeAg positivity was closely related to maternal HBeAg positivity (χ^2=5.82, P<0.05) (Table2). A stratification analysis according to mothers' HBV DNA status showed that the relationship between maternal HBeAg positivity and neonatal HBeAg positivity was statistically significant when maternal HBV DNA was both positive (χ^2=5.14, P<0.001) and negative (*P*=0.001) (Table2).

Maternal HBeAg and Phenotype of MoDC and Cytokine secretion

The expression of the surface markers CD80, CD86, and HLA-DR on MoDC derived from neonates born to HBeAg-positive mothers were similar to those of neonates born to HBeAg-negative mothers (CD80: t=0.151, *P*=0.881; CD86: t=0.373, *P*=0.711; HLA-DR: t=1.104, *P*=0.276) (Table3, Fig.4A). The difference of IL-10, IL-12 and IFN-γ secretion levels between two groups was not statistically significant (IL-10: t=0.906, *P*=0.371; IL-12: t=0.764, *P*=0.450; IFN-γ: t=-0.178, *P*=0.859) (Table3, Fig. 5A).

Maternal HBV DNA and Phenotype of MoDC and Cytokine secretion

MoDC from neonates born to HBV DNA-positive mothers exhibited similar expression

of surface molecules as maternal HBV DNA-negative group (CD80: t=0.401, *P*=0.691; CD86: t=0.373, *P*=0.711; HLA-DR: t=1.522, *P*=0.136) (Table3, Fig.4B). No statistically significant difference was found in the secretion of IL-10, IL-12 and IFN-γ between two groups either. (IL-10: t=1.584, *P*=0.121; IL-12: t=1.896, *P*=0.066; IFN-γ: t=0.178, *P*=0.859) (Table3, Fig. 5B).

Phenotypic analysis showed that expression of CD80, CD86, and HLA-DR on MoDC derived from neonates whose maternal HBV DNA loads were >5×107 copies/ml were significantly lower than those from maternal HBV DNA-negative group (CD80: t=3.238, P=0.002; CD86: t=3.543, P=0.001; HLA-DR: t=2.785, P=0.008) (Table3, Fig. 4C). Compared with maternal HBV DNA-negative group, IL-10, IL-12and IFN-γ secretion were decreased from neonates whose maternal HBV DNA loads were $>5\times10^7$ copies/ml, however there were no statistically significant differences between two groups. (IL-10: t=1.596, *P*=0.119; IL-12: t=1.191, *P*=0.241; IFN-γ: t=0.441, *P*=0.661) (Table3, Fig. 5C).

Allogenic T-Cell Stimulatory Activity of Cultured MoDC

In allergenic MLR, the level of T cell proliferation induced by MoDCs increased with the ratio between DC and T cell-dependent manner. MoDCs from neonates of the HBV intrauterine transmission group had a comparable stimulatory capacity with that from neonates of the HBV intrauterine non-transmission group. The experiment did not

reveal significant differences between MoDCs from neonates of the Maternal HBeAg-positive group and the Maternal HBeAg-negative group in T cell proliferation.

The SI for neonates whose maternal HBV DNA loads were $>5\times10^7$ copies/ml was the lowest when the MLR system contained with 5×10^3 $well^{-1}$ of MoDCs. The results showed that neonates whose maternal HBV DNA loads were $>5\times10^7$ copies/ml tended to have significantly decreased T cell stimulatory activity as compared with the values of maternal HBV DNA-negative group, especially in the cultures at a DC: T cell ratios of 1:5 and 1:10 ($t=-5.442$, $P<0.001$; $t=-2.195$, $P=0{\cdot}042$) (Fig. 6).

Discussion

The precise mechanism of how HBV infections of neonates born to HBV carrier mothers evade the immune response and lead to chronic infection still requires elucidation. Although B and T lymphocytes are the main mediators of immunity, their function is under the control of DCs which is the sentinels of host immune system [12,13]. Recently, it has become evident that many viral immune escape mechanisms specifically target DC fuction [14,15]. However, reports on the role of DCs in HBV infection have focused on adult life, there appears to have few reports in the published literature about alterations of the DC system and associated cytokines during the neonatal period, when mother-to infant HBV transmission may take place. The aim of this study was to characterize the phenotypic and functional alterations of neonatal DCs

derived from CBMCs *in vitro*.

In previous studies, an impaired function of DC was suggested to account for the T and B cell hyporesponsiveness in chronic HBV infection demonstrating the HBV infection of MoDC and a reduced expression of costimulatory molecules leading to impaired T cell allostimulation [16]. In this present study, MoDC derived from HBV intrauterine transmission group showed a lower expression of the surface markers CD80, CD86, and HLA-DR compared to the HBV intrauterine non-transmission group. However, there were no statistically significant differences between two groups (Table 1, Fig. 3A). The results of cytokine measurements by ELISA analysis were shown in Table 1, Fig. 3B. IL-10, IL-12 and IFN-γ in MLR supernatants were comparable in both groups. Our observation cannot yet find the defect of MoDC to be associated with the HBV intrauterine transmission. This is in concordance with the findings of a recent vivo report that frequencies of DC subsets was not decreased by HBV intrauterine transmission [3]. Koumbi et al. also demonstrated that the frequencies of DC subsets in total PBMCs in neonates born to HBsAg-positive mothers were similar to those observed in neonates of healthy mothers [5]. There was only one neonate in HBV intrauterine transmission was HBsAg/HBV DNA double positive in this study, the others were all HBsAg single-positive, which might have resulted from blood contamination during delivery rather than real viral replication inside newborns. Therefore, no significant impairment of MoDC function was found in HBV intrauterine

transmission group applying the current criteria for intrauterine transmission. Further evidence is required to confirm this suggestion.

HBeAg positivity is a sign of active HBV replication. The present study showed that maternal HBeAg positivity was a risk factor for neonatal HBeAg positivity, illustrating that neonates of maternal HBeAg positive have more opportunities to be HBeAg positive, and maternal HBV DNA positivity may enhance the relationship between maternal HBeAg and neonatal HBeAg positivity. It is in concordance with the previous report which suggests that the maternal HBeAg can transverse the placenta barrier impacting antigen-presenting function of neonatal DC [17,18] and maternal HBeAg positivity was a risk factor for HBV intrauterine transmission in neonates [19-21]. Therefore, we originally speculated that maternal HBeAg might influence the phenotype and function of neonatal MoDC.

However, the actual result seems different from our conjecture. Compared with neonates born to HBeAg-positive mothers, the expression of co-stimulatory molecules, cytokine concentrations and allostimulatory capacity were mildly reduced in HBeAg-negative group, however there were no statistically significant differences between two groups. The possible reason is that those who have too high HBeAg loads might have been introduced with intervention measures according to national recommendations in our study, therefore the maternal HBeAg loads might not be

enough to affect the neonates.

Most previous reports studied DC generated in vitro from peripheral blood monocytes demonstrating reduced expression of co-stimulatory molecules, impaired cytokine secretion and lower allostimulatory capacity from chronic HBV carriers [1, 22-24]. In this study, all of these parameters were comparable between maternal HBV DNA negative group and positive group. When maternal HBV DNA loads were $>5\times10^7$ copies/ml, the expressions CD80, CD86, and HLA-DR molecules on MoDCs significantly decreased compared to those in maternal HBV DNA negative group. Furthermore, the T-cell stimulatory activities of MoDCs from maternal HBV DNA loads $>5\times10^7$ copies/ml group were much lower than the MoDCs from maternal HBV DNA negative group in MLR when MoDC and T cell were at a ratio of 1 : 5 and 1 : 10. There was no significant difference between them when MoDC and T cell were at a ratio of 1 : 20. This is in concordance with the findings of the level of T cell stimulatory activity induced by MoDCs increased significantly according to the increase of MoDCs number [12].

Taken together, the present study indicated that there is an immature phenotype and dysfunction of MoDC in the neonates with high maternal HBV DNA loads in comparison with the neonates whose maternal HBV DNA were negative. It was reported that HBV DNA positivity was the decisive indicator for active viral replication,

and the replication levels of virus rapidly increase when maternal HBV DNA loads are $>5\times10^7$ copies/ml, producing much more viral particles which might transverse the placental barrier [18]. Therefore, the function and phenotype of neonatal DCs may be impaired when they have early contact with viral particles. It is necessary to study whether the dysfunction of MoDC is associated with the failure to mount an effective immune response for clearance of HBV and the persistent HBV infection in neonates.

There were not notably alteration of cytokine IL-10, IL-12 and IFN-γ produced by MoDCs from neonates in high maternal HBV DNA loads group in this study. IL-10 was known as an inhibitor of cytokine synthesis, and it was first defined as a Th2 cytokine produced by CD4 cells. IL-10 inhibits T cell activity, which is developed against T cell response and viral infections [25]. IL-12 is an important cytokine to stimulate the proliferation of T lymphocytes, the decrease of IL-12 production was directly attributed to the low T-cell stimulatory of DCs from HBV-infected patients in MLR [26] and IL-10 could inhibit the expression of CD80 and CD86 molecules and suppress the IL-12 production of DCs [12].

Some results of our study seemed different from previous reports on vitro-cultured DC from HBV-infected adults. The reasons might account for these differentiations is the different study population and different maturation protocols for in vitro-cultured DC. To our knowledge, this is the first study of this kind. Previous research findings of our

study group prove that maternal HBV DNA loads >5×10^7 copies/ml might be an influencing factor for the alteration of neonatal DC frequencies. This present study is to further confirm aforementioned conclusions and indicate that the phenotype and function of MoDC from neonates born to Hepatitis B virus-positive mothers might be influenced by maternal HBV DNA loads >5×107 copies/ml in vitro maturation. However, further studies are required to investigate the precise mechanism of how DC play important roles in HBV intrauterine transmission, persistent HBV infection and failure of hepatitis B vaccine.

Conclusion

High level of HBVDNA in the maternal environment might lead to minor phenotypic and functional alterations of MoDC from neonates and subsequent deficits in T-lymphocyte activation may contribute to viral persistence. Thus, MoDC might provide functional and useful tools for future mechanism research regarding HBV intrauterine transmission and failure of hepatitis B vaccine from immunological and epidemiological viewpoint.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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Table 1. Relationship between HBV intrauterine transmission and phenotype of MoDC (%, $\bar{x} \pm s$) and cytokine secretion (pg/ml, $\bar{x} \pm s$)

		surface markers of MoDC (%)			cytokine from MLR culture (pg/ml)		
Characteristic	N	CD80	CD86	HLA-DR	IL-10	IL-12	IFN-γ
HBV intrauterine transmission							
Yes	6	29.20±7.03	38.86±9.55	65.81±15.28	19.40±3.83	29.34±11.07	611.38±133.42
No	42	35.33±9.78	42.79±12.24	68.82±10.87	20.99±7.61	29.69±6.08	665.07±102.82

Table 2 Stratified analysis of association between maternal HBeAg positivity and neonatal HBeAg positivity

Maternal HBV DNA	Maternal HBeAg	Neonatal HBeAg		P value
		Positive	Negative	
Positive	Positive	18	7	P<0.001*
	Negative	0	3	
Negative	Positive	1	3	0.001**
	Negative	1	15	

*Continuity correction χ^2 test.

**Fisher's exact test.

Table 3 Relationship between HBsAg-positive mothers' different HBV serological profiles and phenotype of MoDC (%, $\bar{x} \pm s$)and cytokine secretion (pg/ml, $\bar{x} \pm s$)

Characteristic	N	surface markers of MoDC (%)			cytokine from MLR culture (pg/ml)		
		CD80	CD86	HLA-DR	IL-10	IL-12	IFN-γ
Maternal HBeAg							
Positive	29	33.61±8.67	40.51±11.14	66.54±11.14	19.21±5.36	27.80±6.59	652.49±106.92
Negative	19	34.94±11.10	42.11±14.41	72.63±11.70	21.04±7.98	32.10±5.85	645.65±112.60
Maternal HBV DNA							
Positive	30	32.91±8.30	41.43±11.57	66.86±11.32	19.40±6.22	28.69±5.99	651.23±106.66
Negative	18	37.32±11.26	43.73±12.68	71.08±11.23	23.11±8.33	31.24±7.72	670.24±109.43
Maternal HBV DNA load							
>5×107 copies/ml	23	30.44±6.42*	38.22±8.39*	66.37±9.77*	20.34±7.64	28.46±7.23	666.49±93.09
Negative	18	39.00±10.42	49.32±11.66	74.18±7.67	23.90±6.97	30.97±5.96	680.52±110.42

*compared with Maternal HBV DNA-Negative group, $P < 0.05$

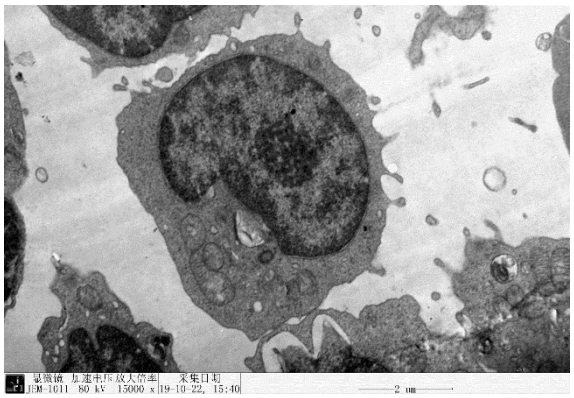


Fig.1 Morphological features of MoDC on 7th day under transmission electron microscope (magnification × 15000).

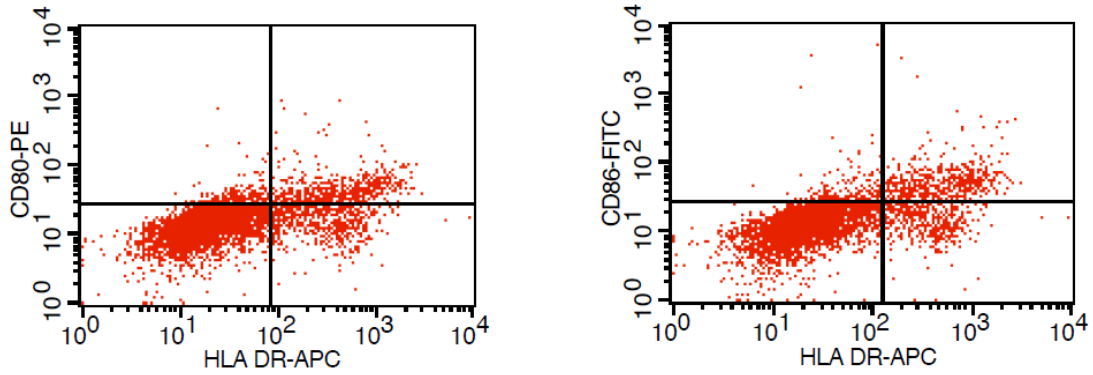
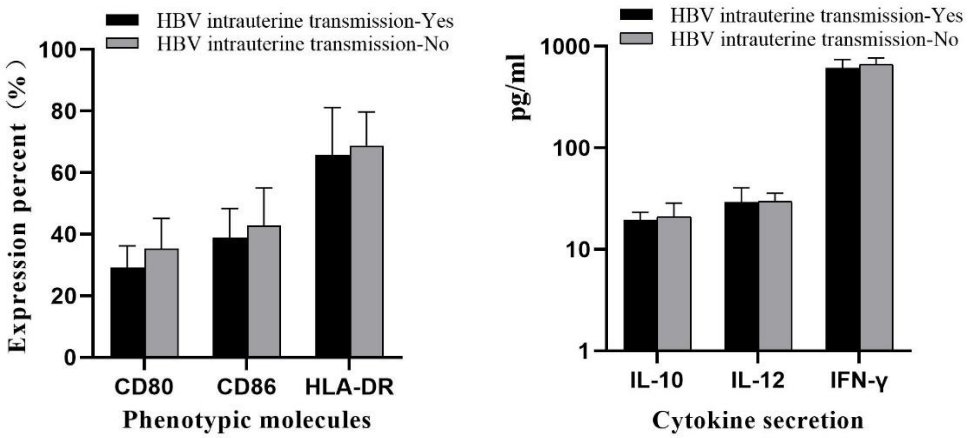
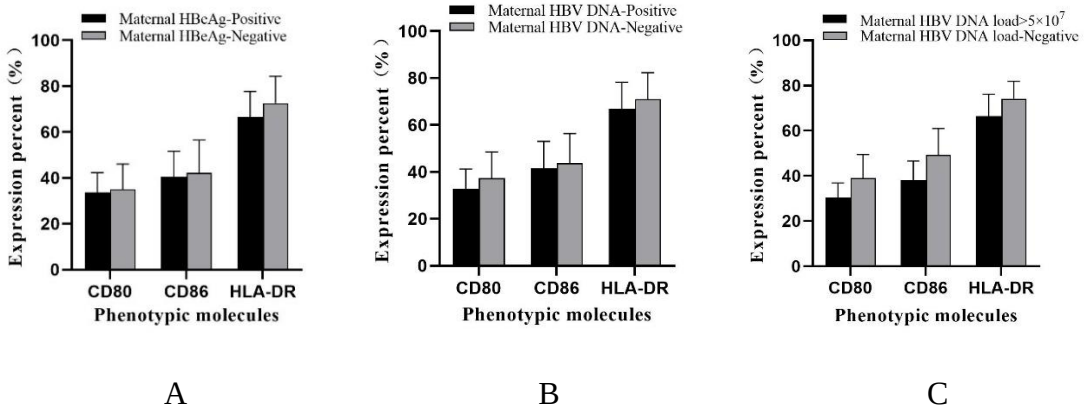


Fig. 2. Expression of surface markers of MoDC from neonates of Hepatitis B virus-positive mothers



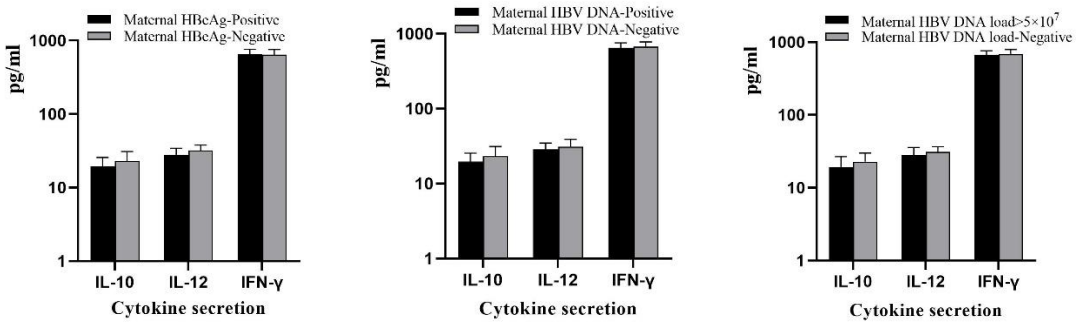
A B

Fig. 3. A, B Comparison of phenotypic molecules and cytokine secretion of MoDC between two groups.



A B C

Fig. 4 A, B, C Comparison of flow cytometric analyses on surface markers of MoDC from neonates of Hepatitis B virus-positive mothers with different HBV serological profiles.



A B C

Fig. 5 A, B, C Comparison of cytokine production of IL-10, IL-12 and IFN-γ in MLR supernatants induced by MoDC from neonates of Hepatitis B virus-positive mothers with different HBV serological profiles.

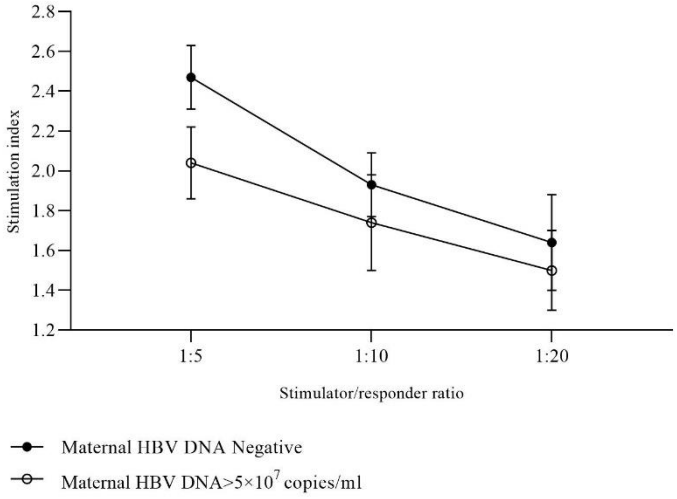


Fig. 6 Allogenic T-Cell proliferation induced by MoDCs was measured in MLR. MoDCs from neonates of maternal HBV DNA loads $>5\times10^7$ copies/ml group exhibit significantly impaired allostimulatory capacity compared to maternal HBV DNA-negative group. Results were significant at stimulator: responder ratios of 1:5 ($p < 0.001$) and 1:10 ($p < 0.05$). The results were expressed as mean ± SD.