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Exploring the Influence of Hepatitis B Immunoglobulin on the Immune Response to the Hepatitis B Vaccine in a Mouse Model

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ABSTRACT

Background: The extent to which maternal antibodies against the hepatitis B surface antigen (HBsAb) acquired transplacentally affect the immune responses to the hepatitis B vaccine (HBVac) in infants is still uncertain.

Objective: To explore the impact of the HBsAb on the immune response to the HBVac in a mouse model.

Methods: According to the doses of the HBVac (2, 5 μg) injected, 267 BALB/c mice were divided into two groups. Each group was subdivided into 3 subgroups based on the doses of the hepatitis B immunoglobulin (HBIG) (0, 25, 50 IU) administered. The HBsAb titers were detected 4 weeks after completing the HepB vaccination.

Results: Among all the mice, 40 had an HBsAb titer <100 mIU/mL (non- or low-response to the HBVac). The rates of the HBsAb titer <100 mIU/mL in 0, 25 and 50 IU HBIG groups were 1.1%, 23.1%, and 20.7%, respectively. Multivariate logistic regression analysis showed that the risk factors for low- or non-response to the HBVac were injection with the HBIG, low HBVac dose, and hypodermic injection. The mean HBsAb titers (log_{10}) reduced gradually in the 0, 25 and 50 IU HBIG groups (P<0.001).

Conclusion: The HBIG administration has negative impacts on the peak level of the HBsAb and the rate of an effective immune response. This implies that the maternal HBsAb acquired transplacentally might inhibit the immune responses to the HBVac in infants.

Keywords: BALB/c mice, HBsAb, Hepatitis B Immunoglobulin, Hepatitis B Vaccine, Immune response

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INTRODUCTION

The hepatitis B virus (HBV) infection still poses a major threat to the global public health [1]. The World Health Organization (WHO) estimates that at least 658,000 individuals die from the HBV-related complications annually, including liver cancer, cirrhosis, and fulminant hepatitis [2]. The HBV-infected young children and infants tend to develop chronic infections, leading to serious outcomes [3, 4]. The Hepatitis B vaccination, in time, is the most affordable control and prevention strategy against the HBV transmission. China has the world's largest burden of the HBV infection and a dramatic reduction has been achieved since the hepatitis B vaccine (HBVac) was integrated into the National Expanded Program on Immunization in 1992 [5].

With the implementation of the HBV vaccination program, more and more women of childbearing age were found to be positive for the HBsAb (around 56-59%) and pregnant women with high HBsAb titers accounted for 21% [6, 7]. Based on the fact that the maternal HBsAb, which in essence belongs to IgG-type antibodies, can be actively transported to the fetus through the placenta, it is predictable that a certain level of the HBsAb antibodies can be detected in growing numbers of neonates at birth [8]. So, it can be inferred that the effectiveness of the HBV vaccination, which is routinely conducted on the 0-1-6 month schedule after birth, may be weakened by the existing maternal HBsAb titers in infants. However, there are only a few studies on the inhibitory effect of the maternal HBsAb on the neonatal hepatitis B vaccination, and the findings are inconsistent [6, 9-11].

Infants are susceptible to the HBV infection if they have no adequate HBsAb titers after a full course of immunization [12]. Therefore, it is necessary to explore whether the maternal HBsAb is one of the inhibitory factors for the effect of the hepatitis B vaccination. However, it poses special ethical challenges to conduct research in neonates due to the high vulnerability of this population. And in China, it is challenging to collect umbilical cord blood or venous blood from neonates after birth and after completing the HBV vaccination. So, to investigate whether the maternal HBsAb titers could suppress the immune responses to the HBVac in infants, we preliminarily designed an animal experiment with BABL/c mice which are universally applied in immunological studies [13, 14].

MATERIALS AND METHODS

Experimental Animals

Two hundred sixty-seven SPF-grade BALB/c mice (128 female and 139 male) with a median age of 6 weeks (interquartile range, IQR, 4-8 weeks) were bred between January 2016 and July 2018. Among them, 136 and 131 were housed at the animal experiment centers of the Affiliated Hospital of Qingdao University (2016) and the Medical College of Qingdao University (2017-2018), respectively. All animals were maintained in a temperature-controlled (22±2 °C) facility with a 12/12-h light/dark cycle and 60%±5% relative humidity. All the mice were given a minimum 7-day acclimation period before starting the experiment. Information about age, gender, the HBVac dose, the hepatitis B immunoglobulin (HBIG) dose, vaccination schedule, and the route was recorded in detail. The mice were purchased from the Jinan Pengyue Experimental Animal Breeding Co. Ltd and standard feedstuff was supplied by the Daren Fucheng Graziery (Qingdao, China).

Grouping and Immunization Schedule

All the mice were assigned to 2 groups (A and B) based on the doses of the HBVac (2, 5 μg) (Hansenula Polymorpha: specification 10 μg/0.5 mL, containing HBsAg 10μg, Hanxin Biological Pharmaceutical, Dalian, China) injected. Each group was subdivided into 3 subgroups (A1-3 and B1-3) based on the doses of the HBIG (0, 25, 50 IU) (specification 200 IU/2mL, Taibang Biological Products,

Shandong, China) administered. The number of mice in each subgroup was listed in Table 1 (A1 43, A2 46, A3 44, B1 48, B2 41, B3 45). And each subgroup contained comparable numbers of male and female mice. The HBIG was administered via intraperitoneal injection, and meanwhile, the HBVac was injected through intramuscular injection (hindquarter muscle) or hypodermic injection (scruff). The profile of the immunization process is shown in Figure 1, and the detailed immunization schedule and the specific numbers of mice contained in each subgroup are shown in Table 1.

Sample Collection and HBsAb Detection

On the 4th week after the third HBVac dose, blood samples were withdrawn from the eye sockets by vacuum tubes without additives [15]. After the blood collection, the mice were immediately sacrificed. Sera were separated within 1 h, placed in a 1.5-ml centrifuge tube, and kept at -80 °C for further analysis of the HBsAb titers.

The HBsAb titers were measured by the chemiluminescent microparticle immunoassay using an ARCHITECT i-2000 fully automated immunoassay analyzer (Abbott Ireland Diagnostics Division). The normal reference value was set as 0-10 mIU/mL. The samples with the HBsAb titer ≧1000 mIU/mL were diluted 1:10, 1:20, 1:30, or below the upper limit of detection (1000 mIU/mL). The HBsAb titers ≧1000, 100-1000,≧100, 10-100, and <10 mIU/mL were considered as high-response (HR), medium-response (MR), protective-response (PR), low-response (LR) and non-response (NR), respectively [12, 16].

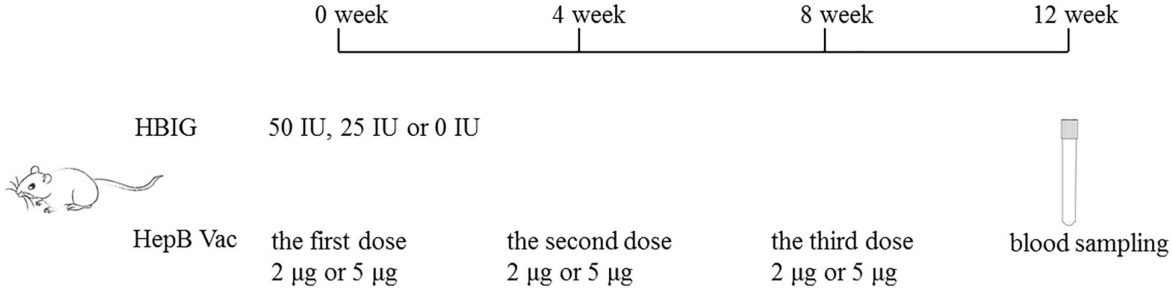

Figure 1. The profile of immunization process

Table 1. Grouping and immunization scheme

Group						Route of HBVac injection	HBIG dose (IU)
A (HBVac 2 ug)			B (HBVac 5 ug)				
Group	n	Sex(n)	Group	n	Sex(n)		
A1	21	Male(11) Female(10)	B1	24	Male (12) Female(12)	IM	25
	22	Male (12) Female(10)		24	Male (12) Female(12)	H	
A2	23	Male (12) Female(11)	B2	21	Male (11) Female(10)	IM	50
	23	Male (13) Female(10)		20	Male (10) Female(10)	H	
A3	22	Male (11) Female(11)	B3	21	Male (11) Female(10)	IM	0
	22	Male (12) Female(10)		24	Male (12) Female(12)	H	

IM, intramuscular injection; H, hypodermic injection. HBIG was administrated by intraperitoneal (IP) injection. HBIG was injected at the beginning (0 week) and three doses of the HBVac were injected at the 0, 4 and 8 weeks respectively.

Statistical Analysis

For categorical variables, the Chi-squared test or Fisher's exact test were utilized for analysis. The HBsAb titers displayed normal distribution after taking logarithms. So, the data were assessed by logarithms and expressed as the mean±standard deviation (SD), and the Student t-test or one-way ANOVA were employed. The risk factors were identified by univariate logistic regression analysis. Significant variables (P<0.1) were then subjected to multivariate logistic regression (MLR) analysis. Statistical tests were performed with the SPSS 22.0, and P<0.05 (two-sided) was deemed statistically significant.

Ethics Approval

All the animal experiments were approved by the Medical Ethics Committee of Qingdao Municipal Hospital and performed in compliance with relevant guidelines and regulations.

RESULTS

The Immune Response Profiles of Mice

Two hundred sixty-seven mice had full immunization, and approximately 41-48 mice were included in each subgroup. Of them, 40 (14.98%) had NR or LR to the HBVac (HBsAb titer ＜100 mIU/mL), including 30 (11.24%) with LR (HBsAb titer 10-100 mIU/mL) and 10 (3.75%) with NR (HBsAb titer ＜10 mIU/mL). In addition, 89 (33.33%) were of MR (HBsAb titer 100-1000 mIU/mL), while 138 (51.69%) were of HR (HBsAb titer≧1000 mIU/mL).

For the specimens containing the HBsAb titer≧1000 mIU/mL, their corresponding HBsAb levels ranged from 10^3 to 10^6 $\log_{10}$ mIU/mL after dilution. Among groups of A1, A2, A3, B1, B2, and B3, the positive rates of HBsAb titers ≧1000 mIU/mL were18.6%, 17.4%, 72.7%, 60.4%, 48.8% and 91.1%, respectively (x^2=77.925, P＜0.001). The HBsAb titer ($\log_{10}$) in each group above was 2.42±1.38 mIU/mL, 2.46±1.01 mIU/mL, 4.17±1.14 mIU/mL, 3.75±1.27 mIU/mL, 3.38±1.11 mIU/mL and 4.52±0.81 mIU/mL, respectively (F=26.253, P＜0.001).

Unfavorable Factors Associated with LR and NR to HBVac

Among the 267 mice, the rates of LR and

Table 2. Risk factors for low- and non-response to the HBVac in mice

Item	n	HBsAb<100 mIU/mL	Univariate analysis		Multivariate analysis	
		n(%)	RR value (95%CI)	P value	RR value (95%CI)	P value
Sex						
Male	139	22 (15.8)	1.149 (0.585, 2.257)	0.687		
Female	128	18 (14.1)	1			
HBIG dose						
0 IU (1)	89	1 (1.1)	1			
25 IU (2)	91	21 (23.1)	26.400 (3.466, 201.112)	0.002	42.970 (5.320, 347.044)	<0.001
50 IU (3)	87	18 (20.7)	22.957 (2.990, 176.230)	0.003	31.613 (3.918, 255.061)	0.001
HBVac dose						
5 μg	134	6 (4.5)	0.136 (0.055, 0.338)	< 0.001	0.093 (0.035, 0.250)	<0.001
2 μg	133	34 (25.6)	1			
Route of HBVac injection						
Intramuscular	132	8 (6.1)	0.208 (0.092, 0.470)	< 0.001	0.139 (0.056, 0.346)	<0.001
Hypodermic	135	32 (23.7)	1			

HBIG: hepatitis B immunoglobulin; HBVac: hepatitis B vaccine

NR to the HBVac were remarkably higher in the HBIG administration groups than in the control group. In addition, the rates of LR and NR were dramatically higher in the 2 μg HBVac group than in the 5 μg HBVac group, and likewise, the rates were also markedly higher in the hypodermic injection group than in the intramuscular injection group. MLR analysis revealed that the HBIG administration, low HBVac dose, and hypodermic injection were independent, unfavorable factors associated with LR and NR to the HBVac in mice (Table 2).

The Effects of the HBIG Administration on the HBsAb Titers After Vaccination

The effects of the HBIG administration on the HBsAb levels in mice intramuscularly injected with the HBVac were determined. We just chose those mice because the intramuscular injection is the routine route recommended for use in clinical settings. The mean HBsAb titers (log_{10}) decreased gradually in the HBIG groups (0, 25, and 50 IU): 5.09±0.36, 4.29±1.07, and 3.97±1.02 mIU/mL, respectively in the HBVac 5μg group (F=8.889, P<0.001); 4.89±0.59, 3.32±1.21 and 2.56±0.85 mIU/mL, respectively in the HBVac 2 μg group (F=37.848, P<0.001). Significant differences in pairwise comparison (B1 *vs* B3, P=0.004; B2 *vs* B3, P<0.001; A1 *vs* A3, P<0.001; A2 *vs* A3, P<0.001; A1 *vs* A2, P=0.008) were observed, except for the comparison between the HBIG 25 IU and 50 IU in the HBVac 5μg group (B1 *vs* B2, P=0.238) (Figures 2, 3).

Favorable Factors Associated with HR to HBVac

The rate of the HR to the HBVac remarkably decreased in the HBIG administration groups compared with the control group (25 IU *vs* 0 IU, P<0.001; 50 IU *vs* 0 IU, P<0.001). Furthermore, the rate of the HR was dramatically higher in the 5 μg HBVac group than in the 2 μg HBVac group, and it was also markedly higher in the intramuscular injection group than in the hypodermic injection group. The MLR analysis revealed that no HBIG administration, high dose of the HBVac, and the intramuscular injection were independent, favorable factors associated with the HR to the HBVac (Table 3).

DISCUSSION

The HBIG, as a purified solution containing high titers of HBsAb antibodies, is derived from plasma donated by individuals immunized with the HBVac [17, 18]. To imitate the fact that infants get HBsAb transplacental from their mothers, we established an animal model via the injection of the HBIG into BABL/c mice. Then, 3 individual doses of the HBVac were given to each mouse at the 0-4-8 weeks. The efficacy of vaccination was assessed to clarify whether the maternal HBsAb titers could affect the immune responses to the HBVac in infants routinely given on the 0-1-6 month schedule.

In this study, a simple and practical mouse model was established based on the following

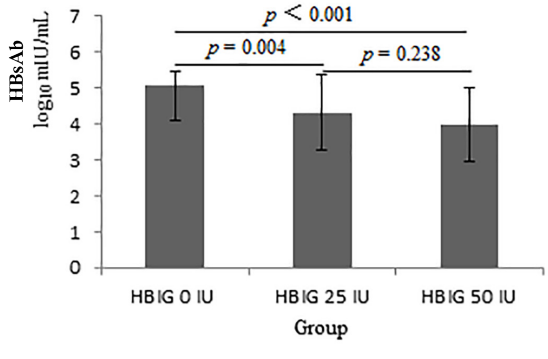

Figure 2. Effect of the HBIG doses on HBsAb levels in mice vaccinated with the 5μg HBVac

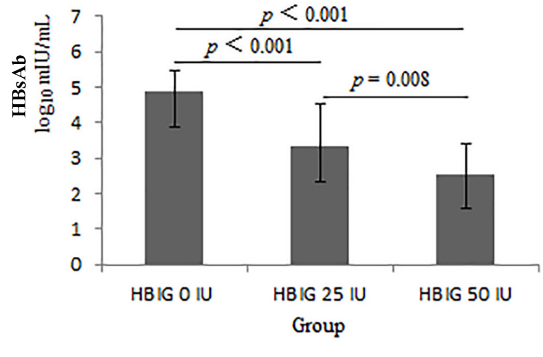

Figure 3. Effect of the HBIG doses on HBsAb levels in mice vaccinated with the 2μg HepBVac

Table 3. Favorable factors related to high-response to the HBVac in mice

Item	n	HBsAb>1000 mIU/mL	Univariate analysis		Multivariate analysis	
		n(%)	RR value (95%CI)	P value	RR value (95%CI)	P value
Sex						
Male	139	75 (54.0)	1.209 (0.747, 1.956)	0.439		
Female	128	63 (49.2)	1			
HBIG dose						
0 IU (1)	89	73 (82.0)	1			
25 IU (2)	91	37 (40.7)	0.150 (0.076, 0.298)	<0.001	0.074 (0.032, 0.173)	<0.001
50 IU (3)	87	28 (32.2)	0.104 (0.051, 0.210)	<0.001	0.049 (0.020, 0.119)	<0.001
HBVac dose						
5 μg	134	90 (67.2)	3.622 (2.186, 6.003)	<0.001	6.707 (3.435, 13.098)	<0.001
2 μg	133	48 (36.1)	1			
Route of HBVac injection						
Intramuscular	132	88 (66.7)	3.400 (2.056, 5.622)	< 0.001	6.727 (3.428, 13.201)	<0.001
Hypodermic	135	50 (37.0)	1			

HBIG: hepatitis B immunoglobulin; HBVac: hepatitis B vaccine

scientific pieces of evidence. First, we adopted a model by injecting the HBIG into young BABL/c mice instead of using vaccinated pregnant mice and their suckling pups, because it is very difficult to draw enough blood for the HBsAb detection from mice, especially suckling mice, without sacrificing them, and also it is quite challenging to vaccinate suckling mice with the HBVac without leading to their death under such stress. Second, in this study, a 0-4-8 week vaccination schedule was conducted based on the pieces of evidence that the lifespan of mice is short and it is recommended that the first HBVac dose should be followed by the second or the third doses with a minimum interval of 4 weeks [19]. Third, in this model, the dosage and proportion of the HBIG 0, 25, or 50 IU and HBVac 2 or 5 μg were used, primarily aiming to simulate the administration of 100 or 200 IU HBIG and 10 or 5 μg of HBVac to prevent perinatal infection in newborns whose mothers were infected with the HBV [6, 20, 21]. A relatively large dose of the HBVac was used to ensure that as many mice as possible could produce a certain amount of antibodies to avoid too many negative cases. Studies on the dose of the HBVac administrated to mice were inconsistent, such as ≦1 μg [22], 2 μg [23], 5 μg [24], and 10 μg [25]. Our results indicated that the dosage and proportion of the HBIG and the HBVac could be further optimized or adjusted according to experimental purposes, which might be a highlight of using this mouse model compared with conducting population-based studies. Fourth, two injection routes for the HBVac administration (hypodermic injection and intramuscular injection) were designed in this study. The reason was that our study aimed to explore a more suitable route for the HBVac injection in mice since different routes were used in some animal experiments [22-26].

In the present study, we demonstrated that the HBIG administration exerted negative effects on the peak level of the HBsAb and rate of immune responses (Table 2, Figures 2, 3), which were in line with our previous findings in neonates receiving a full course of the HBVac [6]. Our results provided evidence supporting the fact that the active immune response of infants could be inhibited by the HBsAb antibodies acquired from their mothers during the HBV vaccination. Another study [9] also found that the antibody response to the HBVac in neonates with high maternal HBsAb titer (>1000 mIU/mL)

was dramatically inhibited after the full course of the HBV vaccination. As for immunoprophylaxis against the vertical HBV transmission, several studies found that the HBsAb titers were lower in neonates who received the HBIG+HBV vaccine at birth than in those injected with the HBV vaccine only [27, 28], indicating that the HBIG might neutralize the HBVac while providing passive immunization for neonates exposed to the HBV. However, other authors did not reveal the relationship between the maternal HBsAb levels and HBVac response in infants [10, 11]. By comparison, some limitations were found in these studies, such as high drop-out rates in the cohort studies, different doses of the HBVac administered, and some discontinuous data from retrospective surveys [9-11], which could be the reasons leading to inconsistent results. Rather, what this study illustrates is the near-term impact of maternal antibodies on the immune response of the HBVac, so it is necessary to discuss whether the long-term immunogenicity of the HBVac is attenuated by the maternal HBsAb.

In addition, we observed that the HBsAb titers were at the levels of 10^3-10^6 $\log_{10}$ mIU/mL when samples with the HBsAb titer ≧1000 mIU/mL were diluted completely. It was reported that long-term maintenance of the HBsAb protecting titer was associated with a high peak level of the HBsAb after vaccination [29, 30]. In this study, those mice with the HBsAb titer ＞1000 mIU/mL are supposed to have a long-lasting response. Therefore, it can be inferred that maternal HBsAb titer is a significant factor influencing persistent antibody responses.

Furthermore, a low dose of the HBVac was found to be an independent, unfavorable factor resulting in LR and NR (Table 2), and a high dose of the HBVac was an independent, favorable factor contributing to the HR (Table 3), indicating that a higher dose of the HBVac injection leads to better immune responses. Therefore, it might be speculated that higher doses of the HBVac were enough to compensate for the neutralization of the maternal HBsAb titers to the HBVac, to activate the immune system of infants and subsequently elicit high HBsAb titers, which was consistent with the conclusions from the study conducted by Hu et al. [9].

Based on the results obtained from this study and previous studies, it is highly recommended that the first HBVac dose should be delayed to prevent the interference of the maternal HBsAb titers. Regarding the specific time for the first dose administration, additional investigation is needed. Previous studies showed that maternally acquired antibody levels decreased rapidly, and then gradually, within the first 4 weeks of life [31], and the passively acquired HBIG was detectable for 4-6 months [32]. Hence, the appropriate time for the first dose administration might be between 1-month and 6-month age or within 1 year after birth. Older infants usually have better responses to vaccines since the immune competence of infants gradually elevates [33]. Agladioglu et al. [34] reported that when the first HBVac dose was delayed until 2 months after birth, the 2-4-9 month schedule produced better immune responses and could provide longer protective effects in comparison with the 0-2-9 month schedule, which was consistent with our suggestion. In China, the first measles vaccine dose is administered to infants at the age of 8 months to prevent the influence of maternally derived antibodies against the measles virus [35, 36]. So, further understanding of the influence of the maternal HBsAb titers on immune responses to the HBVac will facilitate national policymakers to generate a more appropriate schedule for the hepatitis B vaccination.

CONCLUSION

This study established an animal model that imitated the fact that the maternal HBsAb is transferred to infants through the placenta via the injection of the HBIG into BABL/c mice. This model can help make up for the

difficulties in the follow-up of infants, and can also stimulate different levels of the maternal HBsAb titers. The findings indicated that the maternal HBsAb inhibited the immune responses to the HBVac in infants in the short term after a full-course vaccination, and the higher the maternal HBsAb titer was, the greater the inhibitory effect on active immune efficacy of the HBVac was observed. It should be of great concern to further explore whether the maternal HBsAb could interfere with active immunity to the HBVac. This might be a public health threat in the goal of "eliminating viral hepatitis" proposed by WHO in 2016 [37]. To clarify the problem, further research with large sample sizes and longer follow-up periods remain to be carried out in clinical settings.

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Conflict of Interest: None declared.

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