Short Report

Downregulation of IL-12 Production in Healthy Non-Responder Neonates to Recombinant Hepatitis B Vaccine

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ABSTRACT

A proportion of healthy neonates and adults fail to develop a protective antibody response to recombinant hepatitis B (HB) vaccine. Unresponsiveness to vaccination could be attributed to defect in a number of immunological regulatory mechanisms. In this study, IL-12 was quantitated in culture supernatant following *in vitro* stimulation of peripheral blood mononuclear cells isolated from a group of responder and non-responder neonates. Our results indicate significantly decreased production of HBsAg-induced IL-12 in non-responder subjects compared to responders ($P<0.01$). Since IL-12 is produced mainly by antigen presenting cells (APC) and is considered to be crucial for initiation and polarization of CD4+ T-cell function, therefore, our findings could be interpreted to imply APC dysfunction in non-responder vaccinees. *Iran. Biomed. J. 8 (1): 41-45, 2004*

Keywords: IL-12, hepatitis B, vaccination, neonates

INTRODUCTION

Hepatitis B virus (HBV) infection has remained a major public health problem worldwide. Universal vaccination of neonates, particularly in high and intermediate endemic areas, has been proposed by the World Health Organization (WHO) as the most effective strategy for control and eradication of HBV infection [1]. Vaccination with recombinant hepatitis B surface antigen (HBsAg) induces a protective antibody response in the majority of vaccinees. However, 1-10% of both adult and neonate vaccinees fail to respond [2, 3].

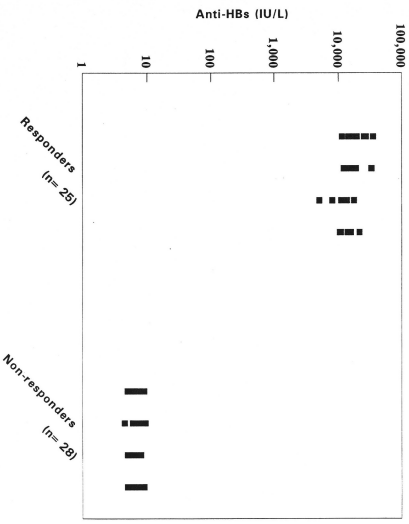
Lack of response to vaccination has been attributed to several immunological mechanisms, including expression of certain HLA antigens and haptotypes leading to defective antigen presentation [4], defects in HBsAg-specific T and/or B-cell repertoires [5, 6], selective destruction of specific B-cells by cytotoxic T-cells (CTL) [7], immuno-logic tolerance [8] and defective T-helper (Th) cell function, either Th1 or Th2, necessary for production of anti-HBs antibody by B-cells [5, 9, 10].

The function of Th cells is initiated and regulated by signals delivered by the antigen presenting cells (APC) and in particular dendritic cells (DC). Thus, impaired APC function could influence the Th1 and Th2 responses [11]. Since IL-12, the heterodimeric cytokine produced mainly by APC and DC is a crucial determinant of Th1 polarization and APC function [12], therefore, quantitation of IL-12 could provide valuable information to elucidate the mechanisms underlying unresponsiveness to HBsAg.

In this study, the level of IL-12 was quantitated for the first time in a group of responder and non-responder vaccinated neonates, following *in vitro* stimulation of peripheral blood mononuclear cells (PBMC) with recombinant HBsAg.

MATERIALS AND METHODS

Subjects and vaccination scheme. Triple doses of 10 µg of a recombinant HB vaccine (Heberbiovac, Heberbiotec Co., Cuba) were administered i.m. to 721 healthy Iranian neonates at 0, 1.5 and 9 months intervals. The first dose was given 24-48 h after delivery in the hospitals. Two to four weeks after completion of the vaccination course, peripheral blood was collected and anti-HBs antibody was quantitated in serum by sandwich ELISA. Collectively, 28 non-responders (anti-HBs<10 IU/L) were identified and 25 high responder neonates (anti-



HBs>10000 IU/L) were randomly selected and included in this study.

Measurement of anti-HBs antibody in serum. Anti-HBs antibody was detected in serum by a sandwich ELISA using a commercial kit (Boehring, Germany). The concentration of the antibody was extrapolated from a standard curve constructed from know concentrations of a standard sample provided by the manufacturer.

In vitro stimulation of PBMC. PBMC were separated from heparinized peripheral blood by Ficoll (Pharmacia, Sweden) gradient centrifugation. After washing in RPMI-1640 medium (Gibco, UK), PBMC was resuspended in a complete culture medium containing RPMI-1640 supplemented with 10% heat inactivated fetal calf serum (Seromed, Germany) and antibiotics, including penicillin (100 U/ml) and streptomycin (100 μg/ml). Cells were then seeded at 1×10^6 cells/ml in a 24-well sterile-tissue culture plate (Nunc, Denmark) in presence or absence of 10 μg/ml of purified rHBsAg [13-15] without vaccine additives (Heberbiovac, Cuba) or 10 μg/ml of phytohemagglutinin mitogen (PHA) (Sigma, USA). Following 72 h incubation at 37˚C in a humidified CO_2 (5%) incubator, culture supernatants were collected and stored in -70˚C until use.

Quantitation of IL-12 in culture supernatant. IL-12 (p 70) was measured by sandwich ELISA using a commercial kit (Biosource, USA). The assay was optimized by titration of the paired capture and detection antibodies as suggested by the manufacturer to determine the optimum concentrations of both antibodies. Accordingly, the capture antibody was coated in polystyrene ELISA plates (Maxisorp, Nunc, Denmark) at 1 μg/ml and the biotinylated detection antibody was employed at 0.4 μg/ml.

Statistical analysis. Differences in cytokine concentration between the two groups of subjects were analyzed by the Mann-Whitney U-test and $P<0.05$ were considered significant.

Fig. 1. Serum levels of anti-HBs antibody in responder and non-responder neonates.

RESULTS

The serum titer of anti-HBs antibody in responder and non-responder neonates was found to be 16740 ± 7555 and 7 ± 1.4 IU/L (mean ± SD), respectively (Fig. 1).

The levels of secreted IL-12 in culture supernatant following *in vitro* stimulation of PBMC with HBsAg, PHA or without stimulation are illustrated in Figure 2. The results are summarized in Table 1.

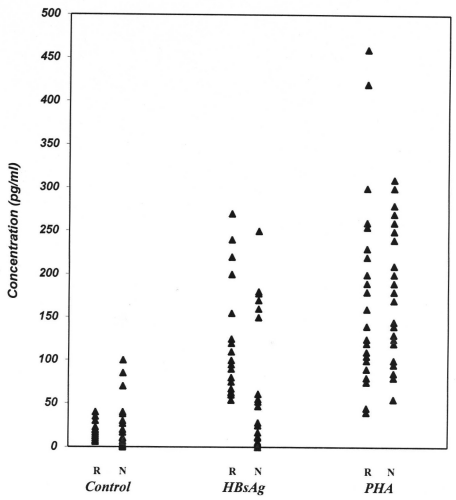
Table 1. Levels of IL-12 secreted *in vitro* from PBMC of responder and non-responder neonates following stimulation with HBsAg, PHA or without stimulation.

Stimulator	Responders	Non-responders	*P* value
HBsAg	109.0 ± 61.5	59.3 ± 76.8	< 0.01
PHA	177.2 ± 106.6	181.2 ± 77.0	NS
Control	25.5 ± 37.6	< 40.2 ± 63.0	NS

* The results represent mean ± SD values (pg/ml); NS: not significant.

Fig. 2. Distribution of IL-12 secreted following *in vitro* stimulation of PBMC from responder and non-responder vaccinees. *R, responders; N, non-responders; Control, culture supernatants from cells cultured in absence of stimulator.

A significant increased production of IL-12 was observed following stimulation of PBMC from responder vaccinees with HBsAg, compared to non-responders ($P<0.01$). Contrary to



HBsAg, no significant differences were found in cytokine levels between the two groups of vaccinees following stimulation with PHA or in absense of stimulation (control).

DISCUSSION

HBsAg is a T-cell dependent glycoprotein and the process of anti-HBs antibody production requires cooperation between T, B and APC [16]. Unresponsiveness to HBsAg, therefore, could be resulted from dysfunction or dysregulation of these cells. We have recently enumerated the HBsAg-specific B-cells in a group of vaccinees and found significantly decreased frequency of specific B-cells in non-responder subjects [6]. Lower frequency of specific B-cells could be attributed to defect in either the primary pre-immune repertoire or the secondary antigen-induced repertoire following vaccination and antigen stimulation. Since determination of frequency of the pre-immune HBsAg-specific lymphocytes has proved to be difficult, due to the low number of naive circulating specific lymphocytes and the limited sensitivity of the assays [6], therefore, assessment of the Th cell and APC function could provide useful information.

To get further insight into the regulatory role of Th function in anti-HBs antibody production, the profile of cytokines secreted by vaccine non-responder adults has been studied by a number of investigators with controversial results, indicating defect in either Th1 or Th2 response [5, 9, 10, 17]. Our recent findings [13], together with those of others [14] indicate defect in both Th1 and Th2 responses in adult non-responder subjects. Similar findings have also been recently observed in non-responder neonates [15]. Impaired Th1 and Th2 function will inevitably influence the B-cell response to HBsAg, leading to lower frequency of specific B-cells reported in non-responder vaccinees [6]. Defective Th1 and Th2 responses could partly be associated to APC dysfunction.

In the present study, we observed significantly decreased production of interleukin-12 (IL-12) by HBsAg-stimulated PBMC from a group of non-responder neonates. The significance of our results is more magnified when analyzed in the context of PHA-induced IL-12 profile. Despite production of higher concentration of IL-12 in response to PHA, as compared to HBsAg, in both responder and non-responder subjects, no significant difference was observed between the two groups (Table 1). These results assure precision of our results and exclude the possibility of involvement of a generalized immune dysfunction in the non-responder subjects or technical shortcomings with regard to *in vitro* stimulation and culture condition. Production of IL-12, a heterodimer cytokine, by APC and particularly DC is considered critical for initiation and polarization of the CD4+ Th response to a variety of intracellular pathogens and malignancies [8, 18, 19]. Recent vaccination trials in experimental animals have revealed that both humoral and cellular immune responses are significantly stimulated by IL-12 [20]. Decreased production of IL-12 has been widely reported in subjects suffering from chronic hepatitis B infection [21, 22]. This has been associated with dysfunction and immature phenotype of DC and accelerated rate of PBMC apoptosis.

Collectively, 5-10% of adult individuals infected with HBV develops chronic carrier state of infection [1]. Similar proportions of healthy neonates and adults fail to develop a protective antibody response to HBsAg vaccine [2, 3]. These overlapping figures suggest that the vaccine non-responder subjects may mostly represent those acquiring chronic infection upon exposure to the wild type virus. Taking this proposition into account, similar immunological regulatory or functional defects are expected to be implicated. Our present results which are reported for the first time in HB vaccine non-responders, together with the previous reports of decreased IL-12 levels in chronic HBV patients, suggest that IL-12 could be viewed as the missing chain in both conditions [21, 22].

These findings propose the potential therapeutic implication of IL-12 in treatment of hepatitis B infection and potentiation of HBsAg-specific response in vaccine non-responders. Based on these propositions, a number of experimental and clinical trials have recently been initiated. The results obtained from these studies confirmed effectiveness of IL-12 in both experimental and transgenic animals [23, 24] and in human [25]. Administration of HB vaccine in chronic HBV-transgenic mice is accompanied with increased production of IL-12 and upregulation of the CD86 costimulatory molecules on DC in vaccine responders but not in non-responders [25], suggesting the importance of DC activation in therapeutic potentiality of HBV vaccine in chronic carriers. Although different mechanisms could be attributed to lack of response to HB vaccine in neonates and adults [3], dysregulation of DC function seems to play a central role in both groups. Since our findings in human neonates are genuine and have not already been reported, therefore, more investigations in both neonate and adult vaccinees are required to verify and consolidate our assumption. We are currently embarking on analysis of IL-12 production and expression of costimulatory molecules on DC from vaccinated adults to extend our findings and explore the mechanisms underlying vaccination failure in human adults vaccinated with recombinant HB vaccine.

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