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IL-1β gene polymorphisms influence hepatitis B vaccination

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Abstract

Considerable variability exists in the vaccine response to hepatitis B with 5–10% of healthy young adults demonstrating no or inadequate responses following a standard vaccination schedule. As the interleukin-1β (IL-1β) cytokine has been shown to be important in the development of immune responses, we determined whether vaccine efficacy is influenced by genetic polymorphisms associated with *IL-1β* expression. Ninety-two healthy individuals who were negative for antibodies to hepatitis B antigen (anti-HBs) were vaccinated against hepatitis B according to a standardized schedule. At selected times, antibody titers and lymphoproliferative capacity to hepatitis B surface antigen (HBsAg) were determined. DNA genotyping for *IL-1β* polymorphisms using a polymerase chain reaction (PCR)–restriction fragment length polymorphism (RFLP) technique demonstrated that both the anti-HBs titer and the T-cell lymphoproliferative response to HBsAg are significantly increased in individuals possessing the *IL-1β* (+3953) minor allelic variant.
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1. Introduction

Hepatitis B is the most common cause of acute and chronic liver diseases and hepatocellular carcinoma. Worldwide, over two billion people have been infected with the virus of whom over 350 million are chronic carriers [1]. Hepatitis B vaccine programs have been implemented effectively for protection against exposure to hepatitis B virus in health care workers and those with occupational risk factors. Experimental and epidemiological studies have demonstrated that not only is there considerable variability in the immune response to hepatitis B surface antigen (HBsAg), but 5–10% of healthy young adults demonstrate no or inadequate responses following a standard hepatitis vaccination schedule [2]. Numerous variables putatively influence vaccination responses including exogenous factors, such as stress, nutrition and infectious disease, as well as endogenous factors, such as gender, age and genetics [3]. Although the contribution that genetic differences play in vaccination failure is unknown, they are believed to be significant [4]. For hepatitis vaccine, the extreme polymorphism and skewed ethnic distribution associated with the HLA multigene family, including both classes I and II alleles, are believed to be a major genetic factor responsible for this variability [2]. HLA molecules are involved in the binding of antigenic peptides and their subsequent presentation to T helper cells [5], thus, regulating the immune response to antigens.

As an alternative to chemical adjuvants, the use of recombinant cytokines as natural adjuvants has been used successfully in several animal species including humans. The adjuvant activity of interleukin-1β (IL-1β) has received the most attention and is believed to act primarily through its ability to increase total blood leukocyte numbers, including $CD8^+$ lymphocytes, and to recruit and stimulate antigen presenting cells involved in humoral immune response, rather than through its inflammatory properties [6,7]. For IL-1β, as well as several other proinflammatory cytokines, there are stable inter-individual differences in secretion levels, and the ability to produce higher or lower cytokine levels cluster in families indicating a genetic basis [8,9]. This is

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due to single nucleotide polymorphisms (SNPs) which have been identified in the regulatory regions of many cytokine genes which affect either the rate of synthesis or stability of mRNA. A variant in the *IL-1β* gene at position +3953 has been described in exon 5 which effects the level of *IL-1β* expression in response to various stimuli and its presence has been associated with a variety of immune and inflammatory diseases, such as insulin-dependent diabetes, psoriasis and periodontal disease [9–12].

These observations led us to hypothesize that the inter-individual variability observed in hepatitis vaccination response is, in part, associated, with the *IL-1β* allele (+3953). Evidence is provided that this SNP is associated with the vigor of antibody and lymphoproliferative responses to HBsAg and influences vaccination efficacy.

2. Methods

2.1. Subjects

Ninety-two healthy volunteers, between 19 and 52 years of age, were recruited from the local university and hospital staff. Each volunteer was fully informed of the procedures and gave written consent before entry into the study. The study was approved by the medical ethical committee of the University Medical Center, Utrecht and the NIOSH/CDC Human Studies Review Board. Personal identifiers and other personal information pertaining to study subjects are not available to CDC personnel. All individuals were tested negative for hepatitis antibodies. Volunteers were vaccinated against hepatitis B (Engerix-B; GlaxoSmithKline Biologicals (GSK), Belgium), by intramuscular administration in the upper arm using a dose of 20 μg recombinant HBsAg according to a 0, 1, and 6 months vaccination schedule. Blood samples were drawn with a vacutainer tube from each volunteer at eight time points; 11 days before vaccination (T_{-11}), at the first vaccination (T_0), 2 weeks after the first vaccination (T_{14}), at the second vaccination (T_{32}), and at 3, 6, 14 and 28 days after the second vaccination (T_{35}, T_{38}, T_{46} and T_{60}, respectively).

2.2. Lymphocyte proliferation

At four time points (T_{-11}, T_0, T_{14} and T_{38}), 50 ml of blood were collected from each volunteer in Vacutainer CPT tubes containing sodium heparin and Ficoll–Isopaque (Becton Dickinson, NJ, USA). Peripheral blood mononuclear cells (PBMCs), obtained by gradient centrifugation, were washed twice with PBS, enumerated and adjusted to 1×10^6 cells/ml in RPMI 1640 culture medium supplemented with 10% inactivated, anti-HBs negative, human AB+ serum, 100 IU/ml penicillin and 100 μg/ml streptomycin. Human PBMCs were cultured in triplicate at a concentration of 4×10^5 cells per well in 96-well round-bottomed culture plates (Greiner 650180, Alphen a/d Rijn, The Netherlands) in triplicate at 37 °C and 5% CO_2. For antigen-specific stimulation HBsAg (GSK, Belgium) was added at a final concentrations of 1, 3 or 5 μg/ml and cultures maintained for 5 days. The cells were then pulsed with 10 μl (1 μCi) of ^{3}H-thymidine (TdR; Amersham Pharmacia Biotech; Buckinghamshire, UK), 20–22 h before harvesting onto glass-fiber filters (LKB-Wallac, Turku, Finland) using a multiple cell culture harvester (LKB-Wallac, Finland) and incorporated radioactivity determined by liquid scintillation counting. Antigen-specific cell proliferation is expressed as the counts per minute (cpm) of [^{3}H]-TdR incorporation in the presence of Ag minus cpm [^{3}H]-TdR incorporation in the absence of Ag.

2.3. Antibody determination

Antibody titers to HBsAg (anti-HBs) were quantified from plasma by microparticle enzyme immunoassay (MEIA) procedure using Axsym© (Abbot Laboratories, Chicago, IL).

2.4. DNA preparation and genotyping

Uncoagulated blood was spotted evenly using a sterile Pasteur pipette onto a clean sheet of filter paper, which was left overnight to dry and stored at room temperature. DNA prepared from freshly obtained human skin samples was used as an internal quality control. Genotyping was performed using a polymerase chain reaction (PCR)–restriction fragment length polymorphism (RFLP) technique as previously described [8,10] with minor modifications [13]. The conditions were: primer sequence: 5′-CTC-AGG-TGT-CCT-CGA-AGA-AAT-CAA-A-3′ and 5′-GCT-TTT-TTG-CTG-TGA-GTC-CCG-3′ 1 μM, 2.5 mM $MgCl_2$; cycling: 95 °C for 2 min followed by 35 cycles at 95 °C for 1 min, 67.5 °C for 1 min, 72 °C for 1 min then a final 5 min at 72 °C. Digestion with 10 units of *Taq*I restriction enzyme at 65 °C for 2 h yielded allele 1 (85 + 97 bp) and allele 2 (182 bp) products. PCR products were electrophoresed into 10% polyacrylamide–TBE gels (Biorad, CA, USA) at 150 V for 30 min and visualized by UV illumination after staining with ethidium bromide.

2.5. Statistical analysis

All analyses were performed using SAS (Version 8, The SAS Institute, Cary, NC). Anti-HBs antibody titers were analyzed using a mixed model analysis of variance with repeated measures over time. Lymphoproliferative responses to HBsAg were analyzed using a split-unit mixed model analysis of variance with repeated measures over time. Significant main effects and interactions were analyzed where appropriate using Student's *t*-tests and are presented as the means. Differences with *P*-values less than 0.05 were considered significant.

3. Results and discussion

Immunizations were conducted in two consecutive winters. A total of 92 individuals were examined and included 55 females and 37 males with a mean age of 22.2 years (median of 21; range of 19–52). The allelic frequency for the *IL-1β* (+3953) polymorphism was comparable to those reported by others and did not deviate significantly from the predicted Hardy–Weinberg distribution [12,13] and included 50 (54%) with 1.1, 37 (40%) with 1.2 and 5 (5.4%) with the 2.2 genotype.

Serum samples were obtained and anti-HBs antibody titers were determined at eight different time points. The antibody titer, expressed as International Units (IU)/l, is used as a reference to establish protection against hepatitis B and is considered effective when the titer is more than 10 IU/l. Since antibody titers decay as a function of time after immunization, an antibody titer of more than 100 IU/l is normally advised. Fig. 1 shows the association between the *IL-1β* variant and anti-HBsAg titers from serum obtained at the day T_{35}, T_{38}, T_{46} and T_{60} time points. Probably due to the weak antigenic properties of HBsAg, specific antibodies were not detected prior to these times (data not shown). As would be expected, a significant increase, independent of the genotype, was observed over the time period examined ($P < 0.0001$). The level of antibody titer increased significantly for the minor variant of the *IL-1β* (+3953) polymorphism compared to allele 1.1 for the time points examined ($P = 0.004$). Although there was an overall gender effect with females demonstrating slightly higher antibody titers than males ($P = 0.042$), there were no significant interactions between gender and gene or gender and time (data not shown).

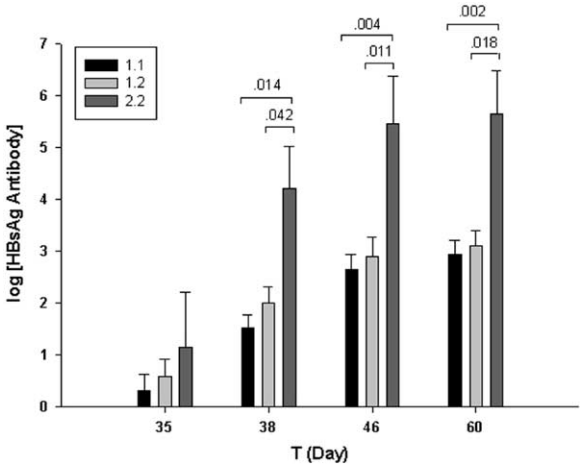

Fig. 1. Mean anti-HBs antibody titers after hepatitis B vaccination. The horizontal axis show the 35, 38, 46 and 60 days time points (*T*). The antibody titers are expressed as International Units/l ± S.E. Numbers appearing above brackets represent difference (*P*-values) between indicated allelic genotype.

Immunity to the hepatitis B vaccination was also determined, in part, by measuring the in vitro proliferative response of PBMCs to HBsAg. Lymphoproliferative responses to HBsAg were first observed 2 weeks following immunization (data not shown). Fig. 2 shows the proliferative responses of PBMCs taken from individuals 38 days after immunization and stimulated with 1, 3 or 5 μg/ml HBsAg. The response was significantly higher in individuals heterozygous or homozygous for the *IL-1β* (+3953) allele.

Recent studies have focused on variations in cytokine levels among individuals and these differences have been

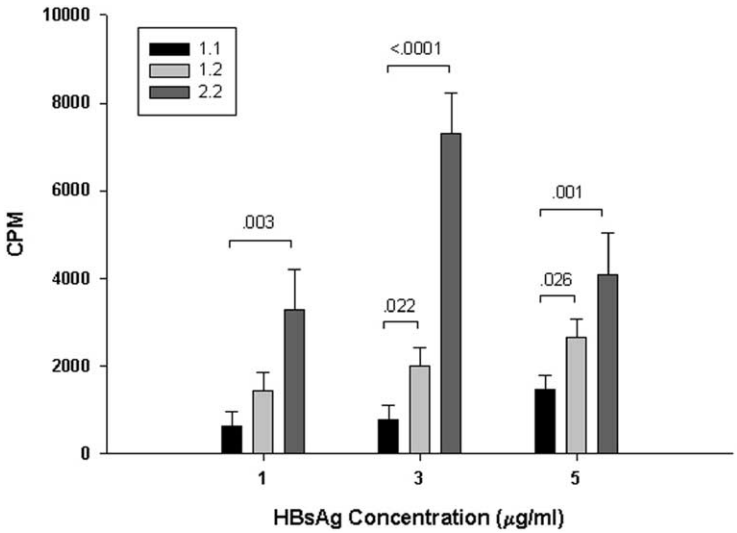

Fig. 2. Mean lymphoproliferative response to HBsAg. Antigen specific cell proliferation is presented as counts per minute (cpm) of [^{3}H]-TdR incorporation in the presence of Ag minus the cpm of [^{3}H]-TdR incorporation in the absence of antigen. The response of PBMCs without HBsAg was 1470 cpm. Numbers appearing above brackets represent *P*-value.

partly ascribed to inheritable SNPs contained within the regulatory elements of cytokine genes [9,14–16]. In this respect, cells from individuals homozygous for the *IL-1β* (+3953) allele and heterozygous cells produce approximately four- and two-fold more IL-1β, respectively, than individuals homozygous for the wild-type allele [8,14]. Higher production of IL-1β has been associated with response to infection, where local induction of these cytokines facilitates the elimination of the microbial invasion [11]. The higher HBsAg immune response associated with the minor *IL-1β* (+3953) variant is consistent with the ability of IL-1β to stimulate B-cell growth and differentiation [6] and increase primary antibody responses [6,17,18]. This immunostimulatory effect of IL-1β, or IL-1β peptide serves as the basis for its increasing use in commercial adjuvant formulations [7]. In contrast to IL-1β, IL-1α does not demonstrate immunostimulatory properties [19] and subsequently we have not observed any significant associations between the *IL-1α* SNP (+4845) and the antibody titer or proliferative response to HBsAg (data not shown). IL-1β also regulates Th1 cytokines, including IL-2 [20], resulting in enhanced T-cell proliferation [21]. This is consistent with the increase in the T-cell proliferative response to HBsAg observed for individuals heterozygous or homozygous for the *IL-1β* (+3953) allele and parallels the serological results.

In conclusion, these studies demonstrate that the immune response to HBsAg is significantly influenced by the minor allelic variant of *IL-1β* (+3953). These effects appear to be sufficient to effect vaccination efficacy, although additional populations should be examined. As the vigor of vaccine responses are under polygenic influences, it is likely that in the near future additional polymorphisms will be identified that influence the immune response to hepatitis as well as other vaccines.

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