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Mannose-binding lectin and ficolin-2 do not influence humoral immune response to hepatitis B vaccine

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Abstract

Background—Host genetics appear to be an important factor in the failure to generate a protective immune response after hepatitis B (HBV) vaccination. Mannose-binding lectin (MBL) and ficolin-2 (FCN2), two pattern recognition receptors of the lectin pathway of complement, influence the clinical outcome of HBV, and MBL deficiency has been shown to augment the humoral response to HBV vaccination in several experimental models. Here, we investigated the association of MBL and FCN2 with the humoral response to HBV vaccination in a candidate gene and functional study.

Patients and methods—A post hoc analysis of a prospective, interventional HBV vaccination study among human immunodeficiency virus type 1 (HIV-1) uninfected individuals in Kenya was conducted. Serum levels and polymorphisms of MBL and FCN2 were analysed in relation to the immune response to HBV vaccination.

Results—Protective hepatitis B surface antibody levels (≥ 10 mIU/ml) were evident in 251/293 (85.7%) individuals. Median MBL and FCN2 levels were similar in responders vs. non-responders with a weak trend towards lower median MBL levels in non-responders (1.0 vs. 1.6 $\mu\text{g/ml}$, $p=0.1$). Similarly, there was no difference in four MBL and six FCN2 polymorphisms analysed in the two groups with the exception of an increased frequency of a homozygous MBL codon 57 mutation in

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non-responders (4 (9.5%) vs. 8 (3.2%), $p=0.05$) corresponding to lower MBL levels. Results were similar after adjusting for age and sex.

Conclusions—Our study does not support a prominent role of the lectin pathway of complement in general and MBL and FCN2 in particular in the humoral immune response to HBV vaccination in African adults.

Keywords

mannose-binding lectin; ficolin-2; hepatitis B vaccination; complement system; innate immunity

Introduction

Hepatitis B virus (HBV) infection remains a major global health concern, with two billion people having been infected worldwide, and 350 million suffering from chronic HBV infection. Each year about 600,000 deaths are attributable to HBV infection as a consequence of chronic hepatitis, cirrhosis, and hepatocellular carcinoma [1]. Chronic infection develops in about 95% of perinatal infected infants and in about 5% of adults that are exposed to HBV, consequently increasing their risk of developing cirrhosis and hepatocellular carcinoma several fold compared to non-carriers [2]. A vaccine against hepatitis B has been available since 1982 and is the mainstay of prevention against later complications of chronic hepatitis B infection. However, vaccination fails to induce protective antibody levels in about 5% of healthy children and adults. Multiple factors that influence vaccine-induced immunity have been implicated including age, gender, immunosuppression, smoking, administration route and host genetics [3, 4]. Importantly, several twin studies suggest the last accounts for 50–75% of the observed variability in antibody responses to a hepatitis B vaccine [5, 6], and recent evidence suggests that several genes of the immune system are linked to variable immune responses to hepatitis B vaccination including members of the complement cascade [7–10], an ancient plasmatic innate defence system that consists of three activation pathways.

The lectin pathway of complement is activated after binding of mannose-binding lectin (MBL) or ficolins (ficolin-1, -2, and -3) to carbohydrate patterns or acetyl groups on pathogens and dying cells or to IgM bound to antigens with subsequent activation of MBL-associated serine protease-1 and -2 and assembly of the C3 convertase [11]. The serum concentration of these pattern recognition receptors (PRR) vary between individuals, with MBL showing the greatest difference (from undetectable to about 10 $\mu\text{g/mL}$) [12], which is caused by polymorphisms in the exon and promoter region of the *MBL2* gene on chromosome 10. Notably, almost a third of the population worldwide displays moderate to severe MBL deficiency [13]. Similarly, a number of major polymorphisms have been described for the ficolin-2 gene (*FCN2*) with a much weaker genotypic/phenotypic relationship than is the case for MBL [14].

Both MBL and ficolin-2 have been implicated in the pathogenesis and clinical outcome of acute and chronic hepatitis B [15–18]. Recent evidence also suggests an important influence of MBL on the humoral response to vaccines [19–22], including hepatitis B vaccine. Ruseva et al. [23] found increased hepatitis B surface antibody (HBsAb) titers in MBL deficient

mice as compared to wild-type mice after intravenous hepatitis B antigen (HBsAg) vaccination, an effect that was abolished after MBL reconstitution. Finally, in a large association study of more than 6000 single nucleotide polymorphisms (SNP), a mutation in the untranslated region of the *MBL2* gene was found to be associated with non-responsiveness to hepatitis B vaccination in Indonesian adolescents and adults [10].

Theoretically, binding of MBL or ficolin-2 to a pathogen or pathogen-associated antigen and subsequent neutralization via the complement system might preclude a response of the adaptive immune system. In case of a vaccine, this might lead to a failure of establishing an adaptive memory and ultimately an insufficient or absent vaccination response. Conversely, MBL deficiency or *FCN2* polymorphisms might facilitate the generation of a protective adaptive immune response to vaccination. However, evidence for an involvement of MBL or the lectin pathway in the immune response to hepatitis B vaccination in humans is lacking. To address this knowledge gap we investigated the association of serum levels and polymorphisms of two PRR of the lectin pathway, MBL and ficolin-2, with the humoral response to hepatitis B vaccination. We hypothesized that the presence of MBL deficiency and polymorphisms in the *MBL2* and *FCN2* genes are associated with a successful response to hepatitis B vaccination in Kenyan individuals.

Patients and Methods

Participants

We conducted a post hoc analysis of a previously published, prospective interventional hepatitis B vaccination study conducted among human immunodeficiency virus type 1 (HIV-1) infected and –uninfected individuals in Thika, Kenya [24]. Briefly, hepatitis B susceptible individuals (negative for HBsAg and HBsAb) received three doses of a hepatitis B vaccine (20 µg of recombinant HBsAg) at 0, 1 to 3, and 6 months. For the current study, data and follow-up serum and whole blood samples from only the HIV-1uninfected individuals only were included. A complete description of the study including detailed information about clinical and laboratory data collection has been reported previously [24, 25].

This study has been approved by the institutional review boards of the University of Washington, the Kenyatta National Hospital, and the Melbourne Health Human Research and Ethics Committee, and all participants had given written informed consent for the study.

Definition of the endpoint

The primary outcome variable for this study was the response to vaccination and was assessed 6 months after completion of the vaccination course by measurement of HBsAb in serum, using the DiaSorin LIAISON anti-HBs II assay (DiaSorin, Saluggia, Italy). Antibody titers were reported as dichotomous outcome according to two cut-offs (10 and 100 mIU/mL, respectively), with titers <10 mIU/mL considered as nonresponse. Frequency of MBL deficiency was the primary predictor of interest. Serum levels and polymorphisms of MBL and ficolin-2 were also analyzed for association with vaccine response.

Assuming a prevalence of *MBL2* exon 1 mutations (hetero- and homozygous) of 50% in the responders (according to previous data from Kenya[13]), power calculations done prior to sample testing estimated 80% power to detect an odds ratio of 3 with a two-sided type I error of 0.05.

Determination of MBL and ficolin-2 plasma levels

Quantification of MBL serum levels was performed by an investigator blinded to vaccine response data using a mannan-binding enzyme-linked immunosorbent assay (ELISA) as previously described [26]. Briefly, mannan-coated microtitre plates were incubated with samples at 1:25 and 1:100 dilutions for 90 min at room temperature followed by detection of bound MBL with a biotinylated monoclonal anti-MBL antibody (HYB 131-01, BioPorto Diagnostics, Denmark). MBL deficiency was defined as serum level < 0.5 µg/mL and, severe as < 0.1 µg/mL, respectively. Ficolin-2 serum levels were quantified using a commercially available ELISA kit (Hycult, The Netherlands).

MBL2 and ficolin-2 genotyping

DNA lysates were prepared from 2µL of stored whole blood according to the manufacturer's instruction (TaqMan Sample-to-SNP, Life Technologies, Australia). Subsequently, *MBL2* and *FCN2* promoter and exon polymorphisms were determined by allele specific polymerase chain reaction (PCR) using TaqMan fluorescent probes (TaqMan genotyping assays, Life Technologies, Australia) as described elsewhere [27]. For assay details, see Supplementary Table 1.

MBL2 genotypes were classified as low (XA/YO, YO/YO), intermediate (XA/XA, YA/YO) or high (YA/YA, XA/YA) producing genotypes according to published literature [28] with exon variant alleles collectively designated as O and the wild-type gene as A, and the promoter variant allele and the wild-type gene designated as X and Y, respectively.

Statistical analysis

We used the chi-square test for comparisons of categorical variables and allele and genotype frequencies and to check for Hardy–Weinberg equilibrium. Logistic regression models with outcome of response to HBV vaccination were used to assess associations with MBL deficiency and various MBL and ficolin-2 polymorphisms. Multivariate logistic regression models, adjusting for age and sex, were also fitted. The Wilcoxon rank-sum test and the Student's t test were used to compare the levels of MBL and Ficolin-2, respectively among responders and non-responders to HBV vaccine.

Haplotype and linkage disequilibrium analysis was carried out with the Haploview program (version 4.2). All other analyses were performed using Stata Intercooled version 11.1.

Results

The study population consisted of 293 HIV-1 uninfected individuals, who received a standard three-dose hepatitis B vaccination course. Median (interquartile range (IQR)) age was 33 years (28–39), and 70 (24%) were female. Protective HBsAb titers (> 10 mIU/mL)

six months after completion of hepatitis B vaccination were evident in 251 (85.7%) individuals. Baseline characteristics were similar in responders and non-responders, as reported previously [24].

MBL and ficolin-2 and response to hepatitis B vaccination

Median (IQR) MBL and ficolin-2 levels were 1.49 (0.44–2.84) $\mu\text{g/mL}$ and 562 (415–767) ng/mL , respectively, and 29% and 16% had moderate and severe MBL deficiency, respectively. Median MBL levels were not significantly different in responders vs. non-responders, as was the frequency of moderate and severe MBL deficiency, although there was a trend towards lower MBL levels in non-responders (Table 1 and Figure 1).

Genotyping was successful in all participants, and *MBL2* and *FCN2* allele frequencies at all positions were in agreement with the predicted Hardy-Weinberg equilibrium (data not shown). Frequency of ficolin-2 polymorphisms observed were comparable to previously reported data from African countries [29] (data from Kenya are lacking). A hetero- or homozygous mutation in the exon1 region of the *MBL2* gene (A/O or O/O) was present in 108/293 (37%) of individuals, an almost 30% lower frequency than previously reported in a Kenyan cohort [13]. In particular, the *MBL2* codon 57 mutation (rs1800451) was encountered less frequently in our study population (minor allele frequency 0.16 vs. 0.24 [13]).

Regarding vaccination response *MBL2* genotype frequencies were similar (low vs. intermediate vs. high producing, Table 1), but there was a trend towards an increased frequency of homozygous exon 1 codon 57 mutations in non-responders (4/42 (9.5%) vs. 8/251 (3.2%), $p=0.05$; minor allele frequency 0.24 vs. 0.15, $p=0.04$ in non-responders and responders, respectively) corresponding to lower MBL levels (Table 1). Results were similar when adjusted for age and sex, and also when using 100 mIU/mL as HBsAb titer cut off to define vaccine response (data not shown).

There was no difference in ficolin-2 serum levels or polymorphisms at the four *FCN2* gene promoter and two exon positions analyzed (Table 2). Results were similar when analyzing *FCN2* genotypes (data not shown).

Discussion

Previously, MBL deficiency and ficolin-2 polymorphisms have been associated with hepatitis B virus infection and disease progression [15–18], and MBL deficiency has been linked to an improved adaptive immune response to vaccination in several experimental models [19, 21, 30] including hepatitis B vaccination [23]. To our knowledge, this is the first study designed to examine the role of two lectin pathway pattern recognition molecules, MBL and ficolin-2, in human hepatitis B vaccine non-responsiveness.

Despite previous experimental studies that were the basis for our *a priori* hypotheses, MBL deficiency or *MBL2* and *FCN2* mutations were not associated with an improved hepatitis B vaccine response in Kenyan HIV-1 uninfected individuals. Rather we found marginal evidence for the opposite to be true, as non-responders were more likely to carry *MBL2*

codon 57 mutations which have been shown to correlate with lower circulating MBL levels [26]. However, these results are limited by the small number of non-responders with homozygous codon 57 mutations (4/42). In line with these genotypic data, there was a trend towards lower MBL levels in individuals who had failed to mount a sufficient antibody response after a standard three dose hepatitis B vaccination course.

Our results are in contrast to most experimental studies demonstrating an augmented humoral response in MBL deficient animals [19, 21, 23]. Several reasons might account for this apparent difference.

First, no single animal model can truly replicate the human immune response to hepatitis B vaccination in all its complexity. In particular, important differences exist in the route of immunization (intravenous/footpad vs. intramuscular) and the antigen and adjuvants used for vaccination in experimental models compared to the human setting. Additionally, the genetic background of animals used might play an important role as outlined in the study by Ruseva et al. [23] Whereas antibody responses were higher in MBL knock-out mice on a mixed genetic background, the opposite was true if backcrossed onto C57BL/6 mice for six or 12 generations. After these genetic manipulations, MBL seems to enhance the humoral immune response to vaccination with HBsAg, which is similar to results from our study.

Second, previous genome-wide association studies [31, 32] failed to identify polymorphisms in the lectin pathway in general or MBL / ficolin-2 in particular associated with response to hepatitis B vaccination in Asian individuals. These data support our results negating a prominent role for MBL or ficolin-2 in the immune response to hepatitis B vaccination in Africans, although we note that data from genome-wide association studies in African individuals are lacking. The earlier result linking an intron SNP in the *MBL2* gene with a high antibody response (>100 mIU/mL) in an Indonesian cohort [10] did not retain statistical significance after correction for multiple testing, and the same SNP was not found to be associated with non-response in a recent genome-wide association study in Chinese Han individuals (although controls were not comparable as a titer > 1000 mIU/mL was chosen in the latter one) [32].

Third, the number of nonresponder cases was rather small and presence of the *MBL2* exon 1 mutation was less frequent as expected [13]. Hence, our study might have been underpowered to detect a small difference.

Interestingly, a recent study in chickens has successfully explored a vaccine preparation that contains an MBL ligand able to inhibit MBL binding and activation of the lectin pathway of complement, in order to achieve a sufficient antibody response after vaccination [33]. A similar approach would certainly be feasible in humans in addition to systemic inhibition of MBL by recombinant human C1 inhibitor [34]. Unfortunately, current data from our study do not support the concept of interfering with binding of HBsAg by MBL or ficolin-2 in order to enable the adaptive immune system to mount a sufficient antibody and memory response, as in our cohort non-responders had even lower MBL levels than responders.

Of note, two very recent studies in individuals after pertussis and pneumococcal vaccination yielded similar results with regards to antibody production and persistence in MBL deficient individuals [35, 36]. However, the influence of ficolin-2 was not investigated.

Limitations of our study include a delayed assessment of response to hepatitis B vaccination 6 months instead of 4 weeks after completion of hepatitis B vaccination. Consequently, this might have had an impact on the categorization of individuals with waning antibody titers as non-responders. In addition, testing for antibodies to hepatitis B core antigen was not performed, which might have facilitated inclusion of individuals with past hepatitis B infection and waning HBsAb response. As the HBsAb assay used in this study only gave a qualitative result, we were not able to correlate MBL or ficolin-2 levels to individual antibody titers. In addition, we limited our analysis to two pattern recognition receptors of the lectin pathway. Ideally, future hepatitis B association studies should include other important lectin pathway proteins like ficolin-1 and -3 and MASP-1 and -2. Importantly, this study was conducted in adults of African ancestry. Hence, our results should not be generalized to individuals of e.g. Asian or Caucasian ethnic background or children.

In conclusion, this study does not support a role of the lectin pathway in general and MBL and ficolin-2 in particular in the humoral immune response to hepatitis B vaccination in African adults.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

Serum mannose-binding lectin levels in hepatitis B vaccination non-responders and responders. Whiskers cap at the 95% level, and horizontal lines inside the boxplot represent medians.

Abbreviations: MBL, mannose-binding lectin

Table 1Association of MBL levels and *MBL2* polymorphisms with hepatitis B vaccination response.

Variables	Responders (n=251)	Non-responders (n=42)	Univariate analysis OR(95% CI)	P-value
MBL levels (µg/ml), median (IQR)	1.6 (0.4–2.9)	1.0 (0.49–2.3)		0.1
<i>MBL deficiency, n%</i>				
None	182 (72.5)	27 (64.3)	Ref	
Moderate (0.1–0.5 µg/ml)	29 (11.6)	8 (19.1)	0.54 (0.22–1.30)	0.17
Severe (<0.1 µg/ml)	40 (15.9)	7 (16.7)	0.85 (0.35–2.08)	0.72
<i>MBL2 exon variants, n (%)</i>				
Codon 57				
G/G	185 (73.7)	26 (61.9)	Reference	
G/A	58 (23.1)	12 (28.6)	0.68 (0.32–1.43)	0.31
A/A	8 (3.2)	4 (9.5)	0.28 (0.08–1.00)	0.05
Codon 54				
G/G	236 (94.0)	39 (92.9)	Reference	
G/A	15(6.0)	3(7.1)	0.83 (0.23–2.99)	0.77
A/A	–	–	–	–
Codon 52				
C/C	239 (95.2)	41 (97.6)	Reference	
C/T	12 (4.8)	1 (2.4)	2.06 (0.26–16.26)	0.49
T/T	–	–	–	–
<i>MBL2 promoter variant Y/X, n (%)</i>				
G/G	186 (74.1)	36 (85.7)	Reference	
G/C	61 (24.3)	5 (11.9)	2.36 (0.89–6.29)	0.09
C/C	4 (1.6)	1 (2.4)	0.77 (0.08–7.13)	0.82
<i>MBL2 genotypes, n (%)</i>				
High producing	158 (63.0)	22 (52.4)	Reference	
Intermediate producing	60 (23.9)	14 (33.3)	0.60 (0.29–1.24)	0.09
Low producing	33 (13.2)	6 (14.3)	0.77 (0.29–2.04)	0.82

MBL2 genotypes were classified as low (XA/YO, YO/YO), intermediate (XA/XA, YA/YO) or high (YA/YA, XA/YA) producing genotypes with exon variant alleles collectively designated as O and the wild-type gene as A, and the promoter variant allele and the wild-type gene designated as X and Y, respectively. Abbreviations: CI, confidence interval; OR, odds ratio; IQR, interquartile range; MBL, mannose-binding lectin;

Table 2Association of ficolin-2 levels and *FCN2* polymorphisms with hepatitis B vaccination response.

Variables	Responders (n=251)	Non-responders (n=42)	Univariate analysis OR (95% CI)	P-value
FCN2 levels (ng/ml), mean (SD)	611.5(312.1)	631.6(385.0)		0.71
<i>FCN2 promoter variants, n (%)</i>				
FCN2 -986, n (%)				
G/G	136 (54.2)	27 (64.3)	Reference	
G/A	99 (39.4)	12 (28.6)	1.64 (0.79–3.39)	0.18
A/A	16 (6.4)	3 (7.1)	1.06 (0.29–3.89)	0.93
FCN2 – 602, n (%)				
G/G	221 (88.1)	40 (95.2)	Reference	
G/A	30 (12.0)	2 (4.8)	2.71 (0.62–11.81)	0.18
A/A	–	–	–	–
FCN2 – 557, n (%)				
A/A	152 (60.6)	25 (59.5)	Reference	
A/G	84 (33.5)	17 (40.5)	0.81 (0.42–1.59)	0.55
G/G ^I	15 (6.0)	0 (0.0)	3.40 (0.54– +inf)	0.22
FCN2 – 4, n (%)				
A/A	151 (60.2)	28 (66.7)	Reference	
A/G	85 (33.9)	12 (28.6)	1.31 (0.64–2.72)	0.46
G/G	15 (6.0)	2 (4.8)	1.39 (0.30–6.42)	0.67
<i>FCN2 exon variants, n (%)</i>				
FCN2 + 6359, n (%)				
C/C	109 (43.4)	19 (45.2)	Reference	
C/T	108 (43.0)	19 (45.2)	0.99 (0.50–1.97)	0.98
T/T	34 (13.6)	4 (9.5)	1.48 (0.47–4.66)	0.5
FCN2 + 6424, n (%)				
G/G	170 (67.7)	28 (66.7)	Reference	
G/T	69 (27.5)	14 (33.3)	0.81 (0.40–1.63)	0.56
T/T ^I	12 (4.8)	0 (0.0)	2.72 (0.43 – +inf)	0.34

^IExact logistic regression was used to compute OR, CI and p-value.

Abbreviations: CI, confidence interval; FCN2, ficolin-2; OR, odds ratio; SD, standard deviation