Travel medicine : knowledge, attitude, practice and immunisation

Roukens, A.H.E.

Intradermal Hepatitis B vaccination in non-responders after topical application of imiquimod (Aldara®)

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Abstract

Background

Five to ten percent of immunocompetent persons fail to develop a protective immune response to hepatitis B vaccination, and are defined non-responders (NR). We investigated the immune response to intradermal hepatitis B vaccination after pre-treatment of the skin with the TLR7 agonist imiquimod.

Methods

Twenty-one non-responders (anti-HBs < 10IU/L after at least 6 intramuscular hepatitis B vaccinations) were randomly assigned to the control group (N=11) or the experimental group (N=10). Participants in both groups received 3 intradermal vaccinations with 5μg HBsAg (0.125mL) at 0, 1 and 6 months. In the experimental group, the dermal site of injection was pre-treated with 250 mg imiquimod ointment. Anti-HBs antibodies were determined at 0, 1, 2, 6 and 7 months.

Results

In both study groups, 70% of the participants developed a protective immune response (anti-HBs ≥ 10IU/L), after the 3rd intradermal vaccination.

Conclusion

The application of imiquimod on the skin prior to intradermal vaccination did not enhance the humoral response to hepatitis B vaccine. However, irrespective of imiquimod application, 70% of the NR who had not responded to 6 previous intramuscular vaccinations, developed a protective immune response with high affinity antibodies after 3 ID hepatitis B vaccinations with 5 μg HBsAg.

Introduction

The immune response to the injected hepatitis B surface antigen (HBsAg) can vary greatly in healthy subjects [1]. Whereas most healthy vaccinees develop an adequate antibody response, defined as an anti-hepatitis B surface antigen (anti-HBs) titre of ≥100 IU/L, five to ten percent of immunocompetent persons fail to develop a protective immune response and never reach an anti-HBs titre exceeding 10 IU/L; these are defined as non-responders (NR) [2]. True NR, defined as NR to 2 series of hepatitis B vaccinations are presumed unlikely to develop adequate anti–HBs titres with further vaccine doses, although no thorough research has been performed to confirm this [3]. The protective efficacy of hepatitis B vaccination is directly related to the induction of anti-HBs antibodies [4-6]. An antibody titre of ≥10 IU/L measured 1–3 months after the administration of the last dose of the primary vaccination series is considered to be a reliable marker of immediate and long-term protection against infection, and those who have an anti-HBs titre of ≥10 IU/L are considered to have protective immunity.
Non-responsiveness to the vaccine has major implications for health care workers and sexual partners of HBV carriers in low endemic countries. In terms of biological mechanisms, non-responsiveness to hepatitis B vaccination has been associated with the presence or absence of specific of MHC alleles. The most pronounced associations with non-responsiveness were with excess of HLA-DR3, -DR7, -DQ2 and -DP11 and with absence of HLA-DR1, -DR5, -DR2, -DQ5 and -DP4 [7-8]. Other characteristics correlated with an inadequate anti-HBsAg response are; higher age, obesity, male gender, and cigarette smoking [9-11].

Several strategies to increase the immune response to the hepatitis B vaccine in NR have been investigated, and comprise of an additional series of standard vaccinations, vaccination of HBsAg combined with other antigens or additional adjuvants [12-14], or alternative routes of administration. The most commonly chosen strategy is to give an additional series (1 to 3 vaccinations) of conventional intramuscular vaccinations, leading to seroconversion in 61% of the revaccinated [15].
Another alternative to enhance immunogenicity in NR would be to administer hepatitis B vaccine in the dermal layer of the skin, instead of injecting intramuscularly. Although the intradermal (ID) vaccination route has shown to elicit slightly lower antibody responses in healthy subjects [16-22], in hepatitis B vaccine low responders (anti-HBs of 10-100 IU/L) and NR, the ID vaccination yielded slightly higher antibody titres compared to intramuscular (IM) vaccination during the first 6 months after vaccination [15,23,24].

In this study, we combined the ID vaccination route with local stimulation of dermal antigen presenting cells as a new approach to obtain a protective antibody response in true hepatitis B vaccine NR. Imiquimod (Aldara®) activates antigen presenting cells (APCs) through the toll-like receptor 7 (TLR7) and is registered for the treatment of (genital) warts and basal cell carcinoma.

Methods

Objectives

This study was conducted to determine whether in NR to hepatitis B vaccination, pre-treatment of the injected skin with a TLR stimulant (Aldara®, one sachet (250mg) applied on 20cm^2 skin) before ID hepatitis B vaccination (5μg; 0·125 mL) would elicit a higher antibody response compared to ID vaccination (5μg; 0·125 mL) without pre-treatment of the skin. Efficacy of vaccination was determined by serum anti-HBs antibody measurement.

Study design and Participants

The protocol and consent forms were approved by the Medical Ethical Committee of the Leiden University Medical Centre (LUMC, The Netherlands; protocol P05.187), and registered in the Dutch Trial Register (#NTR1043). Written informed consent was obtained from each participant.

Healthy volunteers of 18 years and older with a history of at least 2 series of hepatitis B vaccination (one series comprises 3 vaccinations of at least 10µg HBsAg per vaccination) and no postvaccination antibody titre of ≥10 IU/L, were eligible for inclusion. Participants were recruited via the University Medical Centres of Leiden and Utrecht (the Netherlands). Records of previous hepatitis B vaccinations and antibody responses were obtained. We excluded volunteers with a compromised immunity due to underlying illness or immunosuppressive medication, pregnant volunteers and those with (possible) autoimmune disorders. The study was carried out between May 2007 and October 2008. Subjects were randomly assigned to the experimental (with imiquimod pretreatment of the injected skin) or control (without pre-treatment of the injected skin) group. Randomisation was performed with the use of sealed envelopes containing the vaccination code balanced through in permuted blocks of 4.

Hepatitis B vaccine

The hepatitis B vaccine used in this study, HBVAXPRO® 40μg HBsAg/mL, is a recombinant vaccine with alum adjuvant, manufactured by Sanofi Pasteur MSD (Lot

no ND37720) and stored according to manufacturer's guidelines. Multiple dosages (maximally 4) were obtained from one vial for ID vaccination. One ID vaccination of 0·125mL contained 5μg HBsAg.

Vaccination and data collection

At the time of inclusion, data on demographic and clinical characteristics of the participants were obtained. Participants received 0·125mL hepatitis B vaccine (equivalent to 5μg HBsAg) intradermally on the dorsal side of the right forearm at 0, 1 and 6 months. This vaccination site enables careful monitoring of possible adverse events. The quality of the ID injection was defined by the diameter of the arisen cutaneous wheal (adapted from the tuberculin skin test) [http://www.cdc.gov/tb/pubs/Mantoux/part1.htm (accessed 27th of March 2009)], with 6 mm being the lowest acceptable diameter. In the experimental group, a square surrounding 20 cm^2 (equal of 7·9 square inches) was marked on the dosal side of the forearm. The participant applied the content of one sachet of Aldara® (5g, 50mg/g) to the marked surface on the skin. This is the advised dosage per application for the treatment of skin lesions. After the ointment was taken up by the skin (in approximately 3 minutes), the vaccine was injected in the centre of the marked area. The oinment was removed by the participant by washing after 6 hrs. In the control group, the vaccine was administered without pre-treatment of the skin.

Blood samples were collected before vaccination (time point 0), and at 1, 2, 6 and 7 months. In the first blood sample (at time point 0) HBsAg and anti-HBcore antibodies were measured as control for infection with HBV.

Participants were asked to document clinical symptoms (local and systemic) after vaccination in a four-week diary. Solicited symptoms were; erythema, pain and swelling at the site of injection, fever and myalgia. Severity of adverse events was documented as – (absent), +/- (mild), + (moderate) or ++ (severe).

Anti-HBs detection

Anti-HBs titres were assessed by the ARCHITECT Anti-HBs assay (Abbott Laboratories, Chicago, IL, USA) according to the manufacturer's instructions, and were expressed in International Units (IU)/L .

Anti-HBs avidity determination

Avidity of anti-HBs antibodies was measured in duplo by adding 0M (PBS), 2M, 4M and 6M urea to the serum of non-responders who mounted an antibody concentration ≥ 30 IU/l after three intradermal vaccinations (with or without imiquimod). The avidity

index was calculated as the ratio of anti-HBs with 6M urea (dilution 1:1) to anti-HBs in PBS (dilution 1:1). As a control, the avidity index of healthy responders (anti-HBs ≥ 50 IU/l after 3 hepatitis B vaccinations) was measured. The antibody concentration had no effect on the avidity index, measured by diluting serum (2-, 5- and 10-fold) in the presence of 6M urea (data not shown). In five study participants who mounted a protective response anti-HBs avidity was determined longitudinally throughout the course of the 3 intradermal vaccinations, to envisage the process of avidity maturation.

HLA allele determination

Study participants were typed for HLA-DRB1, -DQB1 en DPB1 as described previously [25], in the European Foundation of Immunogenetics (EFI)-accredited HLA laboratory of the Department of Immunology and Haematology, LUMC, the Netherlands. Briefly, DNA was isolated using a commercially semi-automated beads based assay (Chemagen, Baesweiler, Germany). The HLA-DRB and DQB typing was performed with a reversed approach of the PCR/SSOP technique and HLA-DPB1 were determined with a commercially available beads-based hybridization assay (Tepnel, Stanford, CT. USA). The interpretation of the raw data was carried out with computer assisted analysis software [26].

Statistical methods

Power calculations were based on an expected seroconversions (anti-HBsAg titre ≥10 IU/L) of 60% in the experimental group [15] and 10% in the control group. With an α of 0·05 and a β of 0·2, the sample size is 10 participants per group. To take into account a possible lost to follow up of 30%, we aimed to include 13 participants per group.

To correct for skewing of the data, antibody concentrations after experimental and control ID vaccination were log-normalised. Differences between vaccination groups were investigated under the hypothesis that a change from baseline of each endpoint was identically distributed within each of the two vaccination groups against the alternative that median change-from-baseline within one vaccination group differed from the other group. The hypothesis was tested using the non-parametric Wilcoxon's test. Moreover, differences in antibody titres before and after first and second intradermal vaccination with or without imiquimod pretreatment were tested using analysis of variance on the log-transformed data. Where appropriate, Chi-square test or Fisher's exact test was used. Statistical analysis was performed using a computer-assisted software package (SPSS version 14.0, SPSS Inc., Chicago, IL).

Results

Study population

Forty of the 70 non-responders from our database responded to the letter of invitation to participate, of whom 19 chose not to participate due to the travel distance to either of the two university hospitals (Leiden or Utrecht). Ten participants were included in the experimental group and eleven in the control group. One participant in the control group withdrew after the second vaccination due to local pigmentation at the site of vaccination. The data of this person were included in analysis per time point. The total inclusion was stopped at 21 instead of 26, as the calculated number of participants (10 per group) was reached and no further withdrawal occurred. Characteristics of the participants are given in table 1. Hepatitis B surface antigen and anti-HBcore was undetectable in all participants.

Table 1 Demographics and vaccine history of study population

Characteristics	Imiquimod N=10	Control N=11	p-value
Sex (female) (%)	6 (60)	5 (45)	0.5
Age (years) (IQR)	39 (26-52)	36 (23-47)	0.6
BMI (kg m^{-2}) (IQR)	25.4 (22-28)	25.2 (22-27)	0.9
Smoking N (%)	3 (30)	3 (27)	1.0
Total HBsAg (μg) before inclusion (mean) (IQR)	78 (60-75)	62 (60-60)	0.3
Time since last HB vaccination (years) (IQR)	1.7 (0-4)	4.1 (1-9)	0.1
Median anti-HBs titre after last IM vaccination (IU/L) (IQR)	2.3 (0.0-6.0)	2.0 (0.0-5.7)	1.0
Median anti-HBs titre at start trial (IU/L) (IQR)	0.1 (0.0-5.6)	0.0 (0.0-1.0)	0.4

P-values were calculated with χ^2 -test, Fisher's exact test, Wilcoxon or Student's t-test where appropriate. HBsAg = Hepatitis B surface antigen. BMI = Body Mass Index. IM = Intramuscular. IQR = Interquartile range. No significant differences were found between the imiquimod and control group (p-values > 0.1).

Vaccine administration

The mean diameter of the cutaneous wheal that appeared after intradermal vaccination (total N=62) was 10.4 mm, with a range of 8-14 mm.

Topical Imiquimod application and anti-HBs response

The anti-HBs titre was measured at each vaccination and one month after each vaccination. Evaluation of the overall anti-HBs antibody responses before and after

the vaccinations revealed the development of antigen-specific humoral responses after vaccination in most of the volunteers, the overall height of which did not depend on pre-treatment with imiquimod (Figure 1). Boosting of the responses by a third vaccination 6 months after the first two was observed, and again, did not differ between the experimental and control group. Responders with high anti-HBs remained good responders throughout the study, and those with low anti-HBs remained low responders.

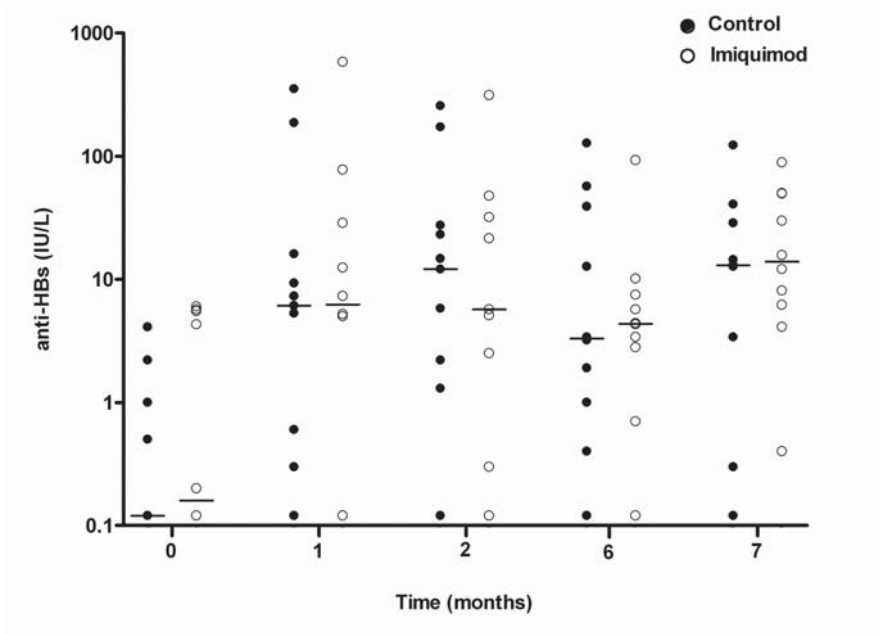

Figure 1 Serologic response (anti-HBs) according to time of vaccination

Intradermal vaccinations were performed at 0, 1 and 6 months. Bars represent medians. Anti-HBs titres of 0.00 were recoded into 0.12 in order to appear in the figure. Each dot represents one participant, dots can overlap.

When a seroconversion of anti-HBs ≥ 10 IU/L was taken as an endpoint, no difference was measured in the time of achievement of this seroconversion in the imiquimod and the control group (figure 2) (Logrank test, $p = 0.8$). One month after the last vaccination, 7 of 10 (70%) of the experimental group and 8 of 11 (73%) of the control group developed an anti-HBs titre ≥ 10 IU/L ($p = 0.9$, Fisher's exact test).

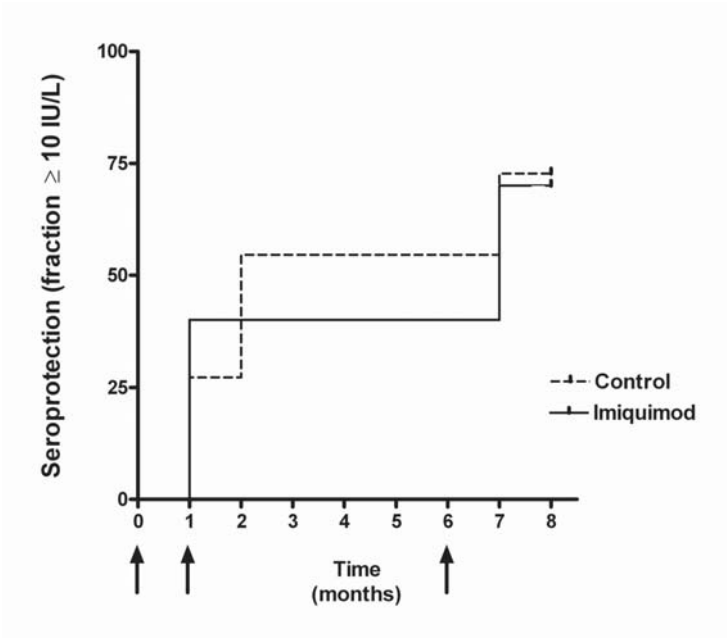

Figure 2 Cumulative percentage of participants achieving an anti-HBs titre of ≥10 IU/L

Arrows indicate intradermal vaccinations. Imiquimod N = 10, control N = 11.

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Avidity of anti-HBsAg antibodies

In healthy responders to the hepatitis B vaccine, avidity maturation occurs during the vaccination series [27]. Since the participants in our study had at least 6 vaccinations to which they did not mount a protective response, we measured the avidity of anti-HBs antibodies of those in whom the additional ID vaccinations did induce an antibody response of ≥ 30 IU/l (N=7). The avidity index (anti-HBs in 6M urea / anti-HBs in PBS) of these previous non-responders was 0.72 (95%CI 0.61-0.93). The avidity index of healthy controls (N=9) in whom 3 intramuscular hepatitis B vaccinations induced an anti-HBs ≥ 50 IU/l was 0.60 (95%CI 0.35-0.85). Anti-HBs avidity after the first ID vaccination did not differ from the anti-HBs avidity after the last ID vaccination (data not shown). Apparently, in this group of previous non-responders, no avidity maturation occurred but instead, the anti-HBs response immediately showed high avidity, comparable to that in healthy vaccinees after a primary vaccination series.

Responding vs. non-responding participants

To gain more insight in the development of a protective serologic response in the study population, several known associations were investigated. These were: sex, age, weight, smoking, and history of hepatitis B vaccination. After multivariate analysis, increased age was associated with non-response in this trial (Table 2).

Table 2 Demographics and history of hepatitis B vaccination of study population according to serologic response after ID vaccination

Characteristics	Responder (≥10 IU/L) N=15	NR (<10 IU/L) N=6	uni variate p-value	multi variate p-value
Sex (male) (%)	5 (33)	5 (83)	0.06	-
Age (years) (mean) (IQR)	31 (22-45)	52 (49-58)	0.001	0.02
BMI (kg m-2) (mean) (IQR)	24.8 (21-27)	26.5 (24-29)	0.4	-
Smoking N (%)	6 (40)	0 (0)	0.1	-
Total HBsAg (μg) before inclusion (mean) (IQR)	64 (60-60)	84 (60-120)	0.4	-
Time since last HB vaccination (years) (IQR)	2.2 (0-3)	4.8 (1-10)	0.1	-
Anti-HBs titre after last IM vaccination (IU/L) (IQR)	2.4 (0.0-6.3)	0.0 (0.0-5.3)	0.5	-
Anti-HBs titre at start trial (IU/L) (IQR)	0.1 (0.0-4.3)	0.0 (0.0-0.0)	0.007	0.6

P-values were calculated with χ^2 -test, Fisher's exact test or Student's t-test where appropriate.
NR = Non-responder. BMI = Body Mass Index. IM = Intramuscular. IQR = Interquartile range.

Distribution of HLA alleles associated with antibody response after Hepatitis B vaccination

Both study groups were comparable with respect to HLA-DR and HLA-DP distribution. Alleles strongly associated with a non/poor response (DR3, DR7, DP11) [9] were present in 13 of 21 participants (6 in the imquimod group and 7 controls); alleles strongly associated with a high response (DR1, DR5, DR2, DPB1*0401) were present in 4 participants (2 in the experimental group and 2 controls). The comparison between the median anti-HBs antibodies titres of the different HLA-groups is reported in table 3.

Adverse events

Participants reported adverse events in a four-week diary after each vaccination. At any time point, the application of imiquimod did not elicit any additional local erythema or tenderness, nor any systemic symptoms, except myalgia after the 1st vaccination (table 4). However, the ID vaccination of the vaccine did induce local

Table 3 Comparison of the peak antibody titre (within one participant during the study) between groups determined by HLA alleles associated with high of low response to hepatitis B vaccination [9]

HLA-DR and -DP allele association with response	Peak antibody titre median (IU/L)	Interquartile range
Non-response (N=13)	15.7	6.2 – 23.1
Neutral (N=4)	39.7	29.5 – 83.8
High response (N=4)	177.1	1.9 – 409.2

Non-reponse: DR3, DR7 and/or DR11, neutral: None of or both the HLA-DR and –DP alleles mentioned, high response: DR1, DR5, DR2, and/or DPB1*0401.

erythema in approximately 80%, swelling in 75% and pruritus in 30% of participants. In 11 of 21 participants, the first and second vaccination were still visible as a pigmented area at the time of the last blood sampling.
Severity of local adverse events was documented as – (absent), +/- (mild), + (moderate) or ++ (severe), according to participants' experience. There was no difference between the control and experimental group in regard to the severity of the adverse events. The participants who reported redness and swelling experienced these events as mild (66%), moderate (32%) and severe (2%).

Discussion

In this study, application of the TLR7 agonist, imiquimod, on the skin prior to intradermal vaccination did not enhance the humoral response to hepatitis B vaccine in previously hepatitis B vaccine NR. However, irrespective of imiquimod application, 70% of these 'true' NR developed a protective immune response after 3 ID hepatitis B vaccinations with 5 μg HBsAg. This is the first study demonstrating the induction of a protective immune response to additional intradermal hepatitis B vaccinations in individuals who did not respond to 6 previous vaccinations. The presence of high avidity antibodies after the first dose suggests that the previous vaccinations did induce the development of a small number of antigen specific lymphocytes, although not enough for a measurable antibody response.

Although the number of participants in this study is low, the 70% seroprotection rate obtained in this group of NR, who were consecutively enrolled without further selection,

Table 4 Adverse events after ID hepatitis B vaccination with or without pre-treatment with imiquimod of the vaccinated skin

Adverse event			Vaccination preceding adverse events (month)					
			0		1		6	
			IMQ	CON	IMQ	CON	IMQ	CON
Local	Erythema	%	90	73	89	73	90	80
		N days (mean)	19	27	20	23	18	20
	Swelling	%	70	73	89	73	70	70
		N days (mean)	10†	25†	15	22	16	19
	Pain	%	20	0	11	9	10	0
		N days (mean)	5	-	10	1	5	-
	Pruritus	%	30	27	33	18	40	20
Systemic	Myalgia	%	40†	0†	22	9	0	0
		N days (mean)	3	-	4	5	-	-
	Fever	%	0	0	0	0	10	0
		N days (mean)	-	-	-	-	1	-

Number of days are calculated for those who reported this adverse event. IMQ = Imiquimod (experimental) group, CON = Control group. † $p<0.05$ with Fisher's exact test or Student's t-test.

strongly suggests that similar protection rates may be reached in unselected groups of NR. Whether the ID route of HBs-antigen delivery is superior to the intramuscular route remains uncertain, as we have not included a control group of participants who received a similar low vaccine dose intramuscularly. The local adverse events induced by the ID delivery of the vaccine (with aluminiumhydroxide as adjuvant) were perceived as mild to severe, and one participant withdrew because of sustained pigmentation at the site of vaccination. The etiology of this adverse event may be due to local granuloma formation in response to the aluminum adjuvant present in the vaccine. No systemic reactions occurred. If ID hepatitis B vaccination is pursued for research or clinical purposes, we suggest to vaccinate in a less visible site than the forearm, e.g. in the shoulder or back.

The lack of a beneficial effect of imiquimod on the immune reactivity to the vaccine in our study was unexpected. Several publications support the local immune boosting effect of imiquimod. For instance, Aldara®, with imiquimod as its active substance, is registered for the treatment of (genital) warts and basal cell carcinoma, and has recently been shown effective in the treatment of vulvar intraepithelial neoplasia (VIN)

[28]. After treatment with imiquimod, the antigen is processed and presented to cells of the adaptive immune system leading to clearance of the virus and subsequent clearance of the lesions [29]. In addition to functional maturation [30], imiquimod induces migration of dendritic cells from the dermis to draining lymph nodes [31,32]. Subcutaneous administration of imiquimod as vaccine adjuvant simultaneously with the antigen of interest, has shown to induce enhanced responses towards the administered antigen [32,34], However, ID vaccination combined with imiquimod as an adjuvant in mice, failed to increase the response towards the injected antigen [33]. In that study, imiquimod was topically applied for 24 hours before vaccination, possibly decreasing the density of local APCs at the time of vaccination. For this reason, we applied the imiquimod ointment immediately prior to vaccination. We assumed that simultaneous administration of imiquimod and antigen would allow for an enhanced antigen presentation, but a poor penetration of imiquimod through the skin may have disturbed this timing. Perhaps a more frequent application of the ointment would have enhanced its effectivity [35]. Secondly, if the hampered immune response in non-responders is due to a defect on the level of T cells or B cells instead of dysfunctional antigen presentation [36], stimulating APC would be less likely to enhance the antibody production. Finally, although in animal models imiquimod (administered either topically or systemically) has demonstrated adjuvant activity in vaccines using antigenic peptides [37], proteins [38], and DNA [39], the immunostimulating effect of imiquimod in clinical setting was most evident in HPV infection [28,40]. It is possible that HPV infection downregulates a specific aspect of the immune reponse, which is specifically upregulated by imiquimod. This would imply that the immunostimulating effect is virus or antigen specific.

The unexpected high rate of seroprotection observed in this study population places the hypothesis of the 'true' non-responder who will never respond to the hepatitis B vaccine into a new perspective. On the other hand, the ID vaccination route has shown to be a potential vaccination route for several vaccine antigens [41] and could also have contributed to the high rate of seroprotection with high avidity antibody response in these NR. The beneficial effect of the ID route of hepatitis B vaccination in NR should now be confirmed in a larger cohort.

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