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Transcriptome profiling in blood before and after hepatitis B vaccination shows significant differences in gene expression between responders and non-responders

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ABSTRACT

Introduction: As the hepatitis B virus is widely spread and responsible for considerable morbidity and mortality, WHO recommends vaccination from infancy to reduce acute infection and chronic carriers. However, current subunit vaccines are not 100% efficacious and leave 5–10% of recipients unprotected.
Methods: To evaluate immune responses after Engerix-B vaccination, we determined, using mRNA-sequencing, whole blood early gene expression signatures before, at day 3 and day 7 after the first dose and correlated this with the resulting antibody titer after two vaccine doses.
Results: Our results indicate that immune related genes are differentially expressed in responders mostly at day 3 and in non-responders mostly at day 7. The most remarkable difference between responders and non-responders were the differentially expressed genes before vaccination. The granulin precursor gene (GRN) was significantly downregulated in responders while upregulated in non-responders at day 0. Furthermore, absolute granulocytes numbers were significantly higher in non-responders at day 0.
Conclusion: The non-responders already showed an activated state of the immune system before vaccination. Furthermore, after vaccination, they exhibited a delayed and partial immune response in comparison to the responders. Our data may indicate that the baseline and untriggered immune system can influence the response upon hepatitis B vaccination.

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1. Introduction

Infection with hepatitis B virus (HBV) causes a broad spectrum of liver diseases, which can evolve into a chronic state and eventually fatal liver disease. Therefore, the WHO recommends universal infant hepatitis B immunization, as well as vaccination of adults at risk for hepatitis B. All current hepatitis B vaccines are composed of hepatitis B surface antigen (HbsAg) and employ a 3- to 4-dose vaccination scheme [1]. Based on current scientific evidence, responders are protected for life and require no additional doses later in life, even if they lose their seroprotection over time [2–4]. However, inter-individual variability in the immune response following HBV vaccination is very high. Furthermore,

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persistent non-responders, 5–10% of the vaccinated population, stay unprotected, even after a completed vaccination schedule (WHO, update April 2017).

Inter-individual variability in vaccine-induced immune responses has been shown to originate from multiple factors, including immune competence, age and genetic background [5,6]. This large variability makes it challenging to evaluate clinical efficacy of vaccines. Determination of whole blood early gene expression signatures has been shown to be an important novel tool to assess the efficacy of vaccines and their potential for long-term immunological protection. Previous gene expression analyses on whole blood following vaccination (e.g. influenza, yellow fever vaccines) have shown characteristic transcriptional profiles that correspond with underlying immunological pathways/mechanisms and the resulting protection [5–9].

Previous microarray gene expression studies were not able to provide a functional classifier to discriminate responders from poor-responders after hepatitis B vaccination (Twinrix vaccine) [10]. This could be due to limitations inherent to microarrays, as not all genes/transcripts are measured [10]. Other techniques like in vitro stimulation studies, flow cytometry and serological assessments, could not reveal the underlying reason why some individuals did not develop HBsAg-vaccine-induced protection [1,4,10–17].

In this study, we set out to use whole blood transcriptomics to obtain a better understanding of the poor response to Engerix-B vaccination in the general population. Gene expression levels after the first Engerix-B dose were determined in 34 individuals with no history of hepatitis B vaccination or infection. We investigated if the short-term gene expression signature after the first vaccine dose correlated well with the immunological response in the vaccine recipients. Using 3′ mRNA-sequencing, we profiled the transcriptome of these individuals before, at day 3 and day 7 after vaccination. In addition, we evaluated if these gene expression signatures could be used to predict the antibody response against hepatitis B surface antigen (anti-HBs). The results presented in this paper provide new insights into why some individuals feature a poor antibody response following Engerix vaccination.

2. Methods

2.1. Study cohort

A total of 34 healthy volunteers (20-29y: 10, 30-39y: 7, 40-49y: 16, 50+y: 1, Table 1) without a history of HBV infection or previous hepatitis B vaccination were included in this study after written informed consent. All volunteers received a diary to log their medication intake or episodes of illness, as these factors could influence the general immune system and the immune response upon vaccination. Volunteers were excluded if the pre-vaccination anti-HBs titer was higher than 2 IU/L. In this study, each volunteer received a hepatitis B vaccine dose (Engerix-B, GSK) on days 0 and 30. The vaccination schedule was further completed on day 365. At days 0 (pre-vaccination), 3, 7 and 60, blood samples were drawn from each volunteer. At days 0 and 60 the absolute numbers of white blood cells (with differentiation in monocytes, lymphocytes and granulocytes), red blood cells and platelets were determined with a hematology analyzer (ABX MICROS 60, Horiba). At days 0, 3 and 7 blood was collected in two PaxGene blood RNA tubes (PreAnalytiX GmbH), which contain a buffer that stabilize the *in vivo* gene expression profile, by minimalizing in vitro RNA degradation. At days 0 and 60, serum was taken for anti-HBs titration. Individuals with anti-HBs levels below 10 IU/L were classified as non-responders whereas individuals with anti-HBs levels above 10 IU/L were considered as responders with a protection for at least 30 years.

2.2. RNA extraction

RNA extraction from PaxGene tubes was performed via a column-based RNA extraction using the PaxGene blood RNA extraction kit (Qiagen). To optimize RNA concentrations, we used the RNA clean & concentrator-5 kit (Zymo research). We verified the RNA quality using the Experion™ (Biorad, Experion RNA StdSens Analysis Kit). No RNA samples had to be excluded based on lower quality.

2.3. 3′ mRNA sequencing

All RNA samples were prepared with the QuantSeq 3′ mRNA-Seq Library Prep Kit FWD for Illumina (Lexogen GmbH) following the standard protocol for long fragments. Resulting cDNA libraries were equimolarly pooled, up to 40 samples for one NextSeq 500 sequencing run (high output v2 kit, 150 cycles, single read, Illumina). RNA isolated from both PaxGene tubes, collected from the same individual at the same time, was sequenced in different runs to avoid systemic bias. This led on average to 8.4 million reads for each sample after quality filtering. The variance between the different sequencing runs was limited, with an average relative standard deviation (RSD) of 12% across all measured genes from the same sample and an average RSD of 3% for highly expressed genes.

2.4. NGS data analysis

The raw sequencing data were preprocessed into a read count table after quality trimming via Trimmomatic and gene mapping using HISAT2. Differential expressed genes were identified based on default DEseq2 parameters, namely a log2fold threshold of 0, and significance cut-off of 0.1 with a Benjamini-Hochberg multiple testing correction. Further we employed gene ontology enrichment analysis and functional enrichment within the human protein–protein interaction (PPI) network. Principle component analysis was used to summarize the gene expression variance. A naïve Bayes classifier was then trained on the first 10 principal components. All reported performance metrics (accuracy, area under the ROC curve) are the results on held-out data within a leave-one-out cross-validation, where each sample is left out of the training data set exactly once and used as a validation sample. All raw and processed data have been submitted to the Gene Expression Omnibus. All custom scripts as well as the read count tables can be found at https://github.com/NDeNeuter/hepb_genexp. For more details about the used programs and settings of all different analysis methods, we refer to the supplementary methods.

Table 1
Overview of the cohort.

	Non-responder (titer ≤ 10 mIU/mL)	Low responder (10 < titer < 100 mIU/mL)	High responder (titer ≥ 100 mIU/mL)
Gender (Male)	5	5	2
Gender (Female)	9	5	8
Mean age (year ± std)	37.93 ± 9.93	36.80 ± 7.92	33.20 ± 10.00

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