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

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, 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.

METHODS

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.

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^{TM} (Biorad, Experion RNA StdSens Analysis Kit). No RNA samples had to be excluded based on lower quality.

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.

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.

Ethical forms

This non-commercial study was approved by the medical ethical committee of the Antwerp University Hospital, Belgium (EC15_19_210).

RESULTS

In this study, 20 out of 34 individuals (59%) developed a significant immunologic level of protection at day 60 after two doses (Table 1). As such, this cohort displayed a much larger portion of non-responders (41%) than estimated by the WHO [18]. Also in other small study cohorts larger proportion of non-responders has been reported [10,19–21]. Ten of the 20 responders had a low but protective response (10 mIU/mL < titer < 100 mIU/mL)(Table 1).

Principal component analysis on complete gene expression dataset

All gene counts derived from responders and non-responders at the three different time points were assessed together. Principal component analysis (PCA) revealed no obvious visual separation of samples according to responder status or sampling time points within the first two principal components (PCs) (Supplementary figure 1). However, all time points from each individual did seem to cluster together on the PCA plot. This indicates that variation caused by the vaccination, the difference between time points per individual, is smaller than the gene expression variation between all individuals.

Pre-vaccination variability

To determine the unvaccinated level of variability between responders and non-responders, we set out the gene expression dynamics of both groups at day 0. Twenty-three genes showed significant differential expression when comparing responders with non-responders and 8 of these genes were related to the immune system (Figure 1, Online table). A group of 16 genes, including interferon induced transmembrane protein 1 (IFITM1), granulin precursor/progranulin (GRN), CD63 and others related to neutrophil degranulation and cell transport, were upregulated in the non-responders group (Figure 1). Another set of 7 genes, including tubby-like protein 4 (TULP4), a substrate-like protein in the E3 ubiquitin ligase complex, featured the reversed profile with higher expression in the responders (Figure 1).

The proteins encoded by these differentially expressed genes (DEGs) were studied within a protein-protein interaction (PPI) network. Six proteins were connected in a local protein network (Supplementary figure 2). GRN, a cell growth regulator, and CXCL5, a small cytokine, are direct interactors and both were downregulated in the responders group. Both proteins play a role in inflammation and host immune defense [22,23]. Moreover, GRN is a protector of regulatory T-cells and is the precursor protein of the fragmented granulins which have positive influence on IL-8 expression and therefore the recruitment of neutrophils [23,24].

The absolute numbers of different blood cells were also determined for each individual at day 0. Figure 2 shows that non-responders had a significantly higher number of granulocytes than responders ($p = 0.017$). This observation is in line with the higher expression level of GRN in non-responders compared to responders. The amount of monocytes and lymphocytes was not significantly different between both groups.

Differentially expressed genes according to responder status at days 3 and 7

In a next step we grouped the data per responder status at both time points to compare the DEGs in separate subsets (Table 2).

Responders

We first compared day 3 with day 0 in the responders group and found 349 DEGs (180 up- and 169 downregulated genes: Online table). Gene ontology (GO) enrichment analysis of these DEGs revealed the involvement of 355 significant GO categories including different immunological processes/pathways (Online table). Table 3 shows the 10 most significant enriched GO categories related to the immune system and processing of vaccine antigens. These data indicate that different immunological pathways have been activated following vaccination. Interestingly, when the DEGs were mapped onto a PPI network, we found that a group of upregulated genes at day 3 were coding for proteins that were all connected to following immune-related terms: immune effector process,

regulation of immune system process and activation of immune response. These terms include B- and T-cell processes and are equal to the 3 most significant categories of the GO enrichment analysis (Supplementary figure 3). In addition two genes of the antigen processing and presentation pathway are significantly upregulated. Beta-2-microglobulin (B2M) helps to present the antigen on the MHC I and is part of the cell-mediated immunity, so not directly related to the antibody production. On the other hand, Cathepsin B (CTSB), a protease that ensures the intracellular degradation and the turnover of proteins into presentable MHC II antigens, is directly related to the humoral immunity, involving CD4+ T-cells, B-cells and the production of antibodies. When comparing day 7 to day 0 in the responders group, we found fewer DEGs (25 down- and 15 upregulated genes) and also fewer enriched genes related to immunological processes (5 DEGs). These data indicate that the impact of Engerix-B vaccination on gene expression levels had reached its peak before day 7 in the responders group. This is similar to other vaccine studies where the vaccination-induced gene expression was highest at day 1 and almost disappeared by day 7 [25–27].

Non-responders

We also evaluated gene expression dynamics in non-responders at days 3 and 7 compared to day 0. At day 3 only 10 DEGs were found of which 3 genes (KCTD7;DCAF12;MARCH8) are immune related (Online table). Those 3 genes were all essential components of the ubiquitination process. KCTD7, a direct interactor with cullin-3 based E3 ubiquitin-protein ligase complex, was the most downregulated DEG in the non-responders at day 3 [28]. There was no significant differential expression of KCTD7 at the other time points and also not in the group of responders. The two other immune related genes (DCAF12;MARCH8) were upregulated and this was also observed to the same extent in the responders group at day 3. DCAF12 was the second most upregulated DEG in the non-responders. It is a substrate for cullin-4 based E3 ubiquitin-protein ligase complex [29]. MARCH8 was the least upregulated DEG and is an membrane-bound E3 ubiquitin-protein ligase [30]. The multifaceted ubiquitination process is known to play a role in antigen processing [31] and from our

data it seems that some components are similarly activated in both non-responders and responders whereas other elements, such as KCTD7 are only downregulated in the non-responders.

At day 7, 155 DEGs were identified. These DEGs represent a large scala of functions in 243 GO categories (Online table, Table 4). One specific DEG from the antigen processing and presenting pathway, namely the TAP binding protein (TAPBP), that is responsible for peptide loading on MHC I, was downregulated and is directly correlated with cell-mediated immunity. When mapping the 155DEGs onto a PPI network, we observed that some upregulated DEGs leaded to leukocyte activation and lymphocyte co-stimulation (Supplementary figure 4). More importantly, we noted a second pattern of upregulated DEGs important for immune effector processes and T-cell mediated immunity. On the other hand, the PPI analysis did not show any specific involvement of B-cell immunity.

Classifier of antibody immune response based on immune gene modules

In a next step we aimed to build a predictive framework that could classify prior to or shortly after vaccination an individual's response as being good or poor. Pre-vaccination 3' mRNA expression data can be used within a machine learning context to judge predictability of these data [26]. PCA was used to define a set of PCs that summarized expression values from different genes. Based on the loadings, we could evaluate possible functional associations for each of the PCs (Table 5) following GO enrichment analysis on genes with the largest contributions (loadings > 0.01 or < -0.01). Based on functional assessments, we chose the first 10 PCs as GO enrichment results decreased within later PCs (Online table). These 10 PCs already represented 40,11% of the data variability. The absence of significant GO enrichments in some PCs indicates that these PCs are likely capturing noise and that there was no functional relationship between the genes that strongly contribute to the principle component. Whereas the PCs represent pre-vaccination gene expression variance, it should be noted that many do assign high loadings to genes related to the immune response. This suggests that

differences within the immune system are a primary source of variation within individual gene expression even without external stimuli.

To determine the strength of gene expression analyses in predicting HBsAg antibody responses following Engerix-B vaccination, we applied a naive Bayes classifier to the first 10 PCs within a leave-one-out cross validation scheme. The classifier achieved a performance on the held-out data with maximum accuracy of 63.6% and area under the ROC curve of 0.662 (Figure 3). This indicates that these gene expression PCs do contain information related to eventual seroconversion. It could correctly identify 30% of responders within the held-out data without any false positives. However, the ROC curve does show a classifier that is unlikely to be useful for clinical practice in its current form, as it has difficulty in classifying the majority of responders/non-responders. When certain immune system related information is added such as the significant absolute granulocytes count, the classifier performance increased with an accuracy of 64,7% and an area under the ROC curve of 0.695 (Figure 3). Other meta-data, like age or gender, did not contribute to the classifier.

DISCUSSION

The aim of this proof of concept study was to evaluate if gene expression studies in blood could distinguish responders from non-responders after de novo hepatitis B vaccination. To measure gene expression changes, we applied a hypothesis-free approach with 3'mRNA sequencing of the transcriptome, a more comprehensive and sensitive technique than microarrays. After two Engerix-B doses, 20 responders and 14 non-responders were identified based on anti-HBs titer measurements. As expected, the overall PCA showed a large inter-individual variation in whole blood gene expression signatures.

The pre-vaccination baseline comparison at day 0 demonstrated a high level of variability in immune-related gene expression between responders and non-responders. This suggests that the individual state of the immune system at the time of vaccination might to some extent determine the effectiveness of Engerix-B vaccination. In this baseline comparison between responders and non-responders, we noticed that expression of the granulin gene (GRN) was significantly upregulated in non-responders. We also found higher amounts of granulocytes in non-responders compared to responders. The precursor granulin (GRN) and the fragmented granulins are part of the neutrophil activation pathway in innate immunity. The smaller granulins are the result of an enzymatic breakdown of the precursor protein by elastase, which is released from the neutrophilic granules. The higher amount of granulocytes and higher expression of GRN in non-responders most likely result in higher production of smaller granulins that are known to stimulate Il-8 expression and recruitment of even more neutrophils [23]. Our data thus indicate that in non-responders the neutrophil activation pathway has a higher activation state compared to responders. The baseline comparison between responders and non-responders also revealed that the IFITM1 gene was downregulated in responders compared to non-responders. Since IFITM1 is essential for immune response signaling, this observation also indicates a higher activation state of the immune system in the non-responders before vaccination. After vaccination however, we observed an upregulation of

IFITM1 in the responder group with a peak at day 3. It remains to be elucidated how this higher activation state of the neutrophil pathway and immune response signaling in non-responders interferes with a proper antibody production following Engerix vaccination.

Overall, responders showed gene expression profiles that reflected the expected B- and T-lymphocyte response to the vaccine. B-cell responses were also found in another recent study by Fourati and colleagues, in which individuals were vaccinated against hepatitis A and B (Twinrix) but also against Tetanus, Diphtheria and Cholera, using microarrays [10]. Similar as in other gene expression studies, the highest level of immune related DEGs in our responders cohort was found on day 3 with a subsequent decrease on day 7 [25–27].

In the non-responders no peak of DEGs was observed at day 3. Only 10 DEGs were noticed at this time point of which 3 (KCTD7;DCAF12;MARCH8) are immune-related and involved in the ubiquitination process. The marked downregulation of KCTD7 in only the non-responders at day 3 is interesting but difficult to interpret since no differences between responders and non-responders were noted for the other two components (DCAF12 and MARCH8) of the ubiquitination process [31]. At day 7, a higher number of DEGs (including different immune related genes) was observed in the non-responders. These data show that the peak immune response in non-responders occurred later than in responders and was also less pronounced. The top 50 GO categories in the non-responders group were mostly related to localization, catabolic and metabolic processes in general. The first ranked immune related GO category was leukocyte activation (66st place, p-value = 5,31E-05, adj. p-value = 0,00220594). Only 4 GO categories out of the top 10 immune related categories found in the responders at day 3, were also significant at day 7 in the non-responders. GO categories related to immune response and antigen processing were not present in the non-responders (Online table). These data indicate that in non-responders only a few immune pathways were activated by hepatitis B vaccination and this in a different time scale and at a different rate.

As there were measurable DEGs shortly before vaccination, we tried to build a predictive framework to distinguish those individuals who responded well from those who responded poorly to Engerix-B. We trained a naive Bayes classifier based on specific immune gene modules, as the whole gene expression data of only 34 individuals had too many variables that would have caused overfitting of every model. Our classifier performed similar to the classifier trained by Fourati et al. which was based on 15 gene probes within a micro-array study. It namely performed better than expected by chance with an AUC in the range of 0.65-0.7. However, the performance of both classifiers remains modest and as such they are currently not yet usable in routine clinical practice. But we believe that the classifiers could be improved by adding more immune relevant data such as the unique HBV T-cell and B-cell repertoires, cytokine assay data and personal HLA profiles. For example, adding the distinctive granulocyte cell counts already improved the accuracy of our classifiers.

In conclusion, our study has shown that immune-related gene expression signatures differ between responders and non-responders after Engerix-B vaccination with a peak response at day 3 in the responder group and a delayed and suppressed response at day 7 in the non-responder group. Our data also suggest that the immune status before vaccination may influence the response to vaccines. Additional studies, preferentially using a similar hypothesis-free approach, are required to confirm this observation. Finally, a more holistic and system biology-based approach will be necessary to provide a predictive framework of classifiers to distinguish responders from non-responders.

COMPETING FINANCIAL INTERESTS

The authors declare no competing financial interests.

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References

[1] Cardell K, Akerlind B, Sällberg M, Frydén A. Excellent response rate to a double dose of the combined hepatitis A and B vaccine in previous nonresponders to hepatitis B vaccine. J Infect Dis 2008;198:299–304. doi:10.1086/589722.

[2] Leuridan E, Van Damme P. Hepatitis B and the Need for a Booster Dose. Clin Infect Dis 2011;53:68–75. doi:10.1093/cid/cir270.

[3] Van Damme P, Ward JW, Shouval D, Zanetti A. 25 - Hepatitis B Vaccines A2 - Plotkin, Stanley A. In: Orenstein WA, Offit PA, Edwards KMBT-PV (Seventh E, editors., Elsevier; 2018, p. 342–374.e17. doi:https://doi.org/10.1016/B978-0-323-35761-6.00025-0.

[4] Safar H, Safar MJ, Ajami A, Khalilian AR, Shams-Esfandabad K, Mirabi AM. Long-term T-cell-mediated immunologic memory to hepatitis B vaccine in young adults following neonatal vaccination. Hepat Mon 2014;14. doi:10.5812/hepatmon.22223.

[5] Nakaya HI, Wrammert J, Lee EK, Racioppi L, Marie-Kunze S, Haining WN, et al. Systems biology of vaccination for seasonal influenza in humans. Nat Immunol 2011;12:786–95. doi:10.1038/ni.2067.

[6] Tan Y, Tamayo P, Nakaya H, Pulendran B, Mesirov JP. Gene signatures related to B-cell proliferation predict influenza vaccine-induced antibody response 2013. doi:10.1002/eji.201343657.Summary.

[7] Querec TD, Akondy RS, Lee EK, Cao W, Nakaya HI, Teuwen D, et al. Systems biology approach predicts immunogenicity of the yellow fever vaccine in humans. Nat Immunol 2009;10:116–25. doi:10.1038/ni.1688.

[8] Li S, Rouphael N, Duraisingham S, Romero-Steiner S, Presnell S, Davis C, et al. Molecular signatures of antibody responses derived from a systems biology study of five human

vaccines. Nat Immunol 2014;15:195–204. doi:10.1038/ni.2789.

[9] Dérian N, Bellier B, Pham HP, Tsitoura E, Kazazi D, Huret C, et al. Early Transcriptome Signatures from Immunized Mouse Dendritic Cells Predict Late Vaccine-Induced T-Cell Responses. PLoS Comput Biol 2016;12:1–17. doi:10.1371/journal.pcbi.1004801.

[10] Fourati S, Cristescu R, Loboda A, Talla A, Filali A, Railkar R, et al. Pre-vaccination inflammation and B-cell signalling predict age-related hyporesponse to hepatitis B vaccination. Nat Commun 2016;7:10369. doi:10.1038/ncomms10369.

[11] Lu Y, Cheng Y, Yan W, Nardini C. Exploring the molecular causes of hepatitis B virus vaccination response: an approach with epigenomic and transcriptomic data. BMC Med Genomics 2014;7:12. doi:10.1186/1755-8794-7-12.

[12] Yan K, Cai W, Cao F, Sun H, Chen S, Xu R, et al. Genetic effects have a dominant role on poor responses to infant vaccination to hepatitis B virus. J Hum Genet 2013;58:293–7. doi:10.1038/jhg.2013.18.

[13] Bauer T, Jilg W. Hepatitis B surface antigen-specific T and B cell memory in individuals who had lost protective antibodies after hepatitis B vaccination. Vaccine 2006;24:572–7. doi:10.1016/j.vaccine.2005.08.058.

[14] Friedrich P, Sattler A, Müller K, Nienen M, Reinke P, Babel N. Comparing humoral and cellular immune response against HBV vaccine in kidney transplant patients. Am J Transplant 2015;15:3157–65. doi:10.1111/ajt.13380.

[15] Hennig BJ, Fielding K, Broxholme J, Diatta M, Mendy M, Moore C, et al. Host genetic factors and vaccine-induced immunity to hepatitis B virus infection. PLoS One 2008;3. doi:10.1371/journal.pone.0001898.

[16] Litjens NHR, Huisman M, Baan CC, van Druningen CJ, Betjes MGH. Hepatitis B vaccine-specific

CD4+ T cells can be detected and characterised at the single cell level: Limited usefulness of dendritic cells as signal enhancers. J Immunol Methods 2008;330:1–11. doi:10.1016/j.jim.2007.10.013.

[17] Roukens AH, Visser LG. Hepatitis B vaccination strategy in vaccine low and non-responders: A matter of quantity of quality? Hum Vaccin 2014;7:654–7. doi:10.4161/hv.7.6.14986.

[18] World Health Organization. Hepatitis B vaccines: WHO position paper, July 2017 - Recommendations. Vaccine 2017. doi:10.1016/j.vaccine.2017.07.046.

[19] Van Der Meeren O, Crasta P, Cheuvart B, De Ridder M. Characterization of an age-response relationship to GSK's recombinant hepatitis B vaccine in healthy adults: An integrated analysis. Hum Vaccin Immunother 2015:00–00. doi:10.1080/21645515.2015.1039758.

[20] Van Der Meeren O, Behre U, Crasta P. Immunity to hepatitis B persists in adolescents 15-16 years of age vaccinated in infancy with three doses of hepatitis B vaccine. Vaccine 2016;34:2745–9. doi:10.1016/j.vaccine.2016.04.013.

[21] Chan PKS, Ngai KLK, Lao TT, Wong MCS, Cheung T, Yeung ACM, et al. Response to booster doses of hepatitis B vaccine among young adults who had received neonatal vaccination. PLoS One 2014;9:1–5. doi:10.1371/journal.pone.0107163.

[22] Park B, Buti L, Lee S, Matsuwaki T, Spooner E, Brinkmann MM, et al. Granulin is a soluble cofactor for toll-like receptor 9 signaling. Immunity 2011;34:505–13. doi:10.1016/j.immuni.2011.01.018.

[23] Zhu J, Nathan C, Jin W, Sim D, Ashcroft GS, Wahl SM, et al. Conversion of proepithelin to epithelins: Roles of SLPI and elastase in host defense and wound repair. Cell 2002;111:867–78. doi:10.1016/S0092-8674(02)01141-8.

[24] Wei F, Zhang Y, Zhao W, Yu X, Liu CJ. Progranulin facilitates conversion and function of

regulatory T cells under inflammatory conditions. PLoS One 2014;9. doi:10.1371/journal.pone.0112110.

[25] Bucasas KL, Franco LM, Shaw CA, Bray MS, Wells JM, Niño D, et al. Early patterns of gene expression correlate with the humoral immune response to influenza vaccination in humans. J Infect Dis 2011;203:921–9. doi:10.1093/infdis/jiq156.

[26] Henn AD, Wu S, Qiu X, Ruda M, Stover M, Yang H, et al. High-resolution temporal response patterns to influenza vaccine reveal a distinct human plasma cell gene signature. Sci Rep 2013;3:2327. doi:10.1038/srep02327.

[27] Obermoser G, Presnell S, Domico K, Xu H, Wang Y, Anguiano E, et al. Systems scale interactive exploration reveals quantitative and qualitative differences in response to influenza and pneumococcal vaccines. Immunity 2013;38:831–44. doi:10.1016/j.immuni.2012.12.008.

[28] Liu Z, Xiang Y, Sun G. The KCTD family of proteins: structure, function, disease relevance. Cell Biosci 2013;3:45. doi:10.1186/2045-3701-3-45.

[29] Lee J, Zhou P. DCAFs, the Missing Link of the CUL4-DDB1 Ubiquitin Ligase. Mol Cell 2007;26:775–80. doi:10.1016/j.molcel.2007.06.001.

[30] Oh J, Shin J, Asthma S, Francisco S. Molecular mechanism and cellular function of MHCII ubiquitination. Immunol Rev 2015;266:134–44. doi:10.1111/imr.12303.Molecular.

[31] Loureiro J, Ploegh HL. Antigen Presentation and the Ubiquitin-Proteasome System in Host-Pathogen Interactions. Adv Immunol 2006;92:225–305. doi:10.1016/S0065-2776(06)92006-9.

Figure legends

Figure 1: The gene expression levels of the resulting 23 DEGS in responders versus non-responders at day 0, before vaccination. The responders data were divided in a high responders group with anti-HBs titer > 100 IU/L and a low responders group with anti-HBs titer >10 IU/L and < 100 IU/L.

Figure 2: The difference in absolute numbers of granulocytes in whole blood samples of responders and non-responders.

Figure 3: Naive Bayes classifier on the first 10 principal components A) Classifier is only build on the gene expression results B) The granulocytes data of each individual were added next to their gene expression results.

Supplementary figure 1: Principal component analysis showed the variation between all individuals, responders (n=20) and non-responders (n=14), based on the first two principal components, at day 0, 3 and 7. There is no clear separation between individuals and/or time points.

Supplementary figure 2: The protein-protein interaction network of resulting DEGs between responders and non-responders at day 0 showed that 6 genes of the total 23 genes were downregulated in the responders compared to the non-responders and were connected in the same protein network. Two genes were upregulated in the responders compared to the non-responders at day 0.

Supplementary figure 3: The protein-protein network of all DEGS of the responders at day 3 compared to day 0 showed an upregulation in immune effector processes and upregulated pathways directly connected to immune response and immune system regulation.

Supplementary figure 4: The protein-protein network of all DEGS of the non-responders at day 7 compared to day 0 showed upregulated pathways directly connected to two different subgroups: A) lymphocyte co-stimulation and leukocyte activation B) Immune effector processes followed by T-cell

388 mediated immunity.