B-Cell Gene Expression and Microbiota Prior Immunization Profile Vaccine Humoral Responsiveness

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Research

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Abstract

Background: The identification of new biomarkers is essential to making it possible to predict the degree of protection following vaccination. Very few human studies have focused on baseline characteristics including microbiota and gene expression to underlie vaccine immune responsiveness. We investigated the host whole-blood transcriptome and microbiome before vaccination, to assess the likelihood of their involvement in an effective MVA-neutralizing antibody response (MVA-Nab) two months later.

Results: We based our analyses on data obtained from a randomized clinical study in which participants (n=10) were vaccinated with the MVA-HIV clade B vaccine (MVA-B). Samples were collected at 2-time points prior vaccination (week-2, w0) to study blood transcriptome. Skin wrap (site of vaccination) and stool microbiota were analysed for diversity and abundance (16S rRNAseq). MVA-neutralizing antibody responses were measured at week 8. The levels of MVA-Nab responses were positively correlated with an abundance of *Eubacterium* in stool and *Prevotella* in skin. The simultaneous investigation of blood transcriptome and host microbiota before vaccination showed that genus diversity and bacterial abundance at that time correlated significantly with the expression of genes involved in B cell development stages. The combination of gene expression and microbiota makes it possible to forecast strong responders to MVA-B vaccination.

Conclusion: To our knowledge, this is the first study integrating host blood gene expression and microbiota before vaccination to predict the intensity of humoral response months later. The genes identified are involved in B cell differentiation might open an avenue of research in this field to optimize vaccination strategies.

Background

The largest fraction of immune cells is found at sites colonized by microorganisms, such as the skin or the gastrointestinal (GI) tract [1]. The gut microbiome is essential for the development, maturation, and adequate functioning of the immune system [1, 2]. The human skin is colonized during the postnatal period by microorganisms that prevent the invasion of external pathogens. Crosstalk between these commensals and the immune system is necessary to trigger innate and adaptive immune responses. Increased attention to the relation between the gut commensal bacteria and host immune responses has led scientists to question whether these microorganisms affect the efficacy of vaccines [3, 4]. Moreover, although the composition of fecal microbiota may be one of the multiple factors that modulate host responses to external immunization, little is known about its role in the interindividual disparity in vaccine efficacy.

The first observation of a potential link between the microbiome and vaccines occurred in an oral vaccination model that used a heat-labile enterotoxin from *Escherichia coli* as an adjuvant [5, 6]. In that situation, depletion of the intestinal microbiota was associated with a profound depression of antigen-specific Th1 and Th17 lymphocytes. Similarly, high antibody responses to the seasonal trivalent

influenza vaccine (TIV) and polio vaccine (IPOL) require the presence of intestinal commensals [7]. Inversely, impaired in microbiota composition and diversity have been reported to attenuate immune responses to vaccines [8]. Interestingly, in human infants receiving hepatitis B, diphtheria, tetanus, and *Haemophilus influenza* type B vaccines, a randomized placebo-controlled double-blind trial demonstrated that vaccine-specific immune responses were enhanced by probiotics [9, 10]. Recently described cross-reactivity between gut microbiota antigens and naive and memory $CD4^+$ T and B cells [11] suggests that the antibody response to HIV-1 immunization may be shaped by intestinal B cells stimulated by host commensals [12]. The microbiota is known to be required for a mature B-cell compartment [13]. For example, germ-free mice have abnormalities in their B-cell systems and lower IgA levels than colonized animals of the same genotype [14]. Microbial antigens and microbial metabolites, such as short-chain fatty acids, strongly promote plasma cell differentiation at mucosal and systemic sites [15]. These microbial metabolites promote IgA production by regulating the metabolism and gene expression in B cells in mice models and in *in vitro* study of human B cells [15, 16]. This IgA appears to orchestrate the beneficial mutualism established between the host and gut commensal microbiome by interacting directly with microbiota species.

Presentation of microbial antigens by the different MHC genotypes also contributes to modifying the IgA repertoires, which in turn modulate the composition of the microbiota in the gut [17]. Accordingly, the depletion of anti-inflammatory microbial species and an expansion of proinflammatory species have been observed in human selective IgA deficiency [18]. A lack of intestinal microbial stimulation results in fewer IgA^+ plasma cells in the gut and a lower abundance of IgA in mouse models [19–21]. Thus the diversity of IgA on the mammalian intestinal surface matches the intestinal taxa diversity [22]. For these reasons, host microbial profiling during vaccine administration to might help optimize the vaccine responses and improve the tolerability of multiple antipathogen treatments. The microbiota, after all, constitutes a constant source of natural adjuvants capable of activating a multitude of pathways that control innate and adaptive immunity [23].

Systems biology has been successfully used to investigate the fundamental innate immune mechanisms orchestrating protective adaptive responses after the perturbation of vaccination against yellow fever [24, 25], HIV [26], Ebola [27], and influenza [28]. An important challenge, however, is to analyze individual baseline human health characteristics to help identify those at higher risk of infection despite vaccination. Until now, only a few studies have looked for candidate traits associated with vaccine responsiveness and partially predicting the humoral response to vaccination against influenza [29–32]. No study has examined the interrelations between each individual's immunological state, their microbiota at baseline, and the impact of both on their vaccine-induced immune responses. As the most successful vaccines act through the production of antibodies [33], identifying specific individual characteristics at baseline should enhance our ability for dividing vaccinees into “high responders” or “low responders” [34]. Such predictive markers might serve as a potential diagnostic tool that assists vaccine development by taking into account the interindividual heterogeneity of immune responses.

This study used a systems biology approach to investigate the volunteers' immune predisposition to respond to MVA-B vaccination, assessed by their blood transcriptome profile; specifically that related to their B cell differentiation stages, and its conditioning by the human microbiota before vaccination. That is, we investigated the host gene expression in blood by a microarray approach and the skin and stool microbiota by using 16S ribosomal RNA sequencing both before vaccination. The objective was to examine their potential involvement in an effective MVA-B neutralizing antibody (Nabs) response during the CUTHIVAC 03 randomized phase Ib clinical study. This trial immunized 10 HIV seronegative subjects aged from 18 to 45 years by the intramuscular route with MVA-HIV clade B vaccine. We analyzed their baseline transcriptomic signature and baseline bacterial abundance and diversity in skin and stool to assess their potential association with the intensity of the Nabs response.

Material And Methods

Skin and feces sampling

For each individual, skin swab samples from the deltoid muscle region (~ 5–20 cm below the vaccine administration site) were collected before the vaccination (w0). Skin samples were collected with Catch-All™ Sample Collection Swab kits moistened with SCF-1 solution. The skin surface was sampled for 30 seconds by firmly swabbing the cotton tip back and forth ~ 50 times. The cotton tip was stored in sterile tubes with MoBio solution at -80°C until DNA extraction. Fecal samples for each participant were collected in sterile fecal collection tubes the day before the vaccination, matching the skin sample time points. All samples were stored at 4-5°C until their reception at the IMPACTA clinical trial site, where they were cryopreserved at -80°C. All samples were shipped on dry ice to the IrsiCaixa AIDS Research Institute for DNA extraction, amplification, and sequencing.

DNA extraction and amplicon sequencing from skin and fecal samples

DNA extraction was performed with the DNA Extraction kit from Epicentre Technologies© (Madison, WI, USA). Six aliquots of buffer solution from the DNA extraction kit were used as negative controls. To amplify the variable V3-V4 region from the 16S rRNA gene, we used the primer pair described in the MiSeq™ rRNA Amplicon Sequencing protocol, which already has the Illumina adapter overhang nucleotide sequences added to the 16S rRNA V3-V4-specific primers, i.e., 16S_F 5’-(TCG GCA GCG TCA GAT GTG TAT AAG AGA CAG CCT ACG GGN GGC WGC AG)-3’ and 16S_R 5’-(GTC TCG TGG GCT CGG AGA TGT GTA TAA GAG ACA GGA CTA CHV GGG TAT CTA ATC C) -3’. Amplifications were performed in triplicate 25-µL reactions, each containing 2.5 µL of non-diluted DNA template, 12.5 µL of KAPA HiFi HotStart Ready Mix (containing KAPA HiFi HotStart DNA Polymerase, buffer, MgCl2, and dNTPs, KAPA Biosystems Inc., Wilmington, MA, USA), and 5 µL of each primer at 1 µM. Thermal cycling conditions consisted of an initial denaturation step (3 minutes at 95 °C), followed by 30 cycles of denaturation (30 seconds at 95 °C), annealing (30 seconds at 55 °C), and extension (30 seconds at 72 °C). These were followed by a final extension step of 10 minutes at 72 °C. Once the desired amplicon was confirmed in 1% agarose gel

electrophoresis, all three replicates were pooled and stored at -30 °C until the sequencing library was prepared. After amplified DNA templates were cleaned up for non-DNA molecules and Illumina sequencing adapters and dual indices attached with the Nextera XT Index Kit (Illumina, Inc.), the corresponding PCR amplification program was run, as described in the MiSeq 16S rRNA Amplicon Sequencing protocol. After a second round of cleanup, amplicons were quantified with the Quant-iT™ PicoGreen® dsDNA Assay Kit (Invitrogen, Carlsbad, MA, USA) and diluted in equimolar concentrations (4 nM) for further pooling. Sequencing was performed on an Illumina MiSeq™ platform (Illumina, Inc.) according to the manufacturer's specifications to generate a median of 30,644 paired-end sequences of ~ 300 bp length in each direction (~ 61,289 reads per sample).

Sequence quality control and microbiota analyses

The quality of MiSeq raw sequences was assessed with the FastQC software [35] (http://www.bioinformatics.babraham.ac.uk/projects/fastqc/). Sequences were trimmed with Trimmommatic [36], with a cutoff value of Q30 for both ends, a minimum mean threshold of Q20 for 30-bp-sliding window across sequences, and a minimum read length of 250 bp (**Supplemental Figs. 1a and b**). After quality control, 28 samples including controls (n = 8) and volunteers (n = 10, 5 women and 5 men) for skin and stools, were further analyzed. Mothur pipeline [37] was used to bin 16S rDNA sequences into operational taxonomic units (OTUs) with a threshold of 97% sequence similarity. OTUs present in only a single sample were discarded. Rarefaction curves were represented by defining the maximum subsampling size as the number of sequences of the sample with the fewest sequences (2751 sequences for skin samples, and 1059 sequences for stool samples) (**Supplemental Fig. 1c**). Richness and diversity indexes were estimated by using the summary.single module implemented in mothur. For taxonomical analysis, 16S rDNA sequences were classified according to the GreenGenes database [38] version 13.5.99.

MVA-GFP Neutralizing antibody assay

Anti-MVA neutralizing activities were evaluated in serum collected at week 8 (w8) with an assay based on GFP detection by flow cytometry [39, 40]. It used HeLa cells as targets and a recombinant strain of MVA expressing the enhanced Aequoriae GFP [41]. Serial dilutions of heat inactivated serum were performed in 96-well round-bottom tissue culture plates (TPP, Zurich, Switzerland) containing DMEM (Gibco, Invitrogen) supplemented with 2% fetal calf serum (PAA, Laboratories GmbH, Pashing, Austria). MVAeGFP was then added to each well at a MOI of 0.25. The plate was then incubated for 1 hour at 37 °C until the addition of 1×10^5 HeLa cells. The incubation then continued for an additional 16 hours at 37 °C, 0.5% CO_2. After trypsinization, the cells were washed with PBS supplemented with 0.5% fetal calf serum and 2 mM EDTA and fixed with 2% formaldehyde. GFP expression was analyzed with FACSCanto II and Diva software (BD Biosciences). The percentage of neutralization was defined as the ratio of the reduction in the number of GFP-expressing cells to the number of GFP-expressing cells in untreated control wells.

RNA extraction and data preprocessing for transcriptomic analysis

Whole blood samples of 2.5 mL were collected in PAXgene RNA tubes (PreAnalytix) twice from each volunteer two weeks before (w-2) and the day of the vaccination (w0). These tubes enable the preservation and stabilization of RNA (storage at -80 °C). Total RNA was extracted from whole blood according to the instructions in the handbook accompanying the PAXgene blood RNA Kit (PreAnalytiX, Hombrechtikon, Switzerland). RNA purity and integrity were assessed on the Agilent 2100 Bioanalyzer with the RNA 6000 Nano LabChip reagent set (Agilent, Palo Alto, CA, USA). Samples for microarray hybridization were prepared as described in the Affymetrix GeneChip WT PLUS Reagent Kit User Manual (Affymetrix, Inc., Santa Clara, CA, USA). For hybridization (to Affymetrix Human Gene 2.1 ST Array Plates), washing, staining, and scanning took place in an Affymetrix GeneTitan system, controlled by the Affymetrix GeneChip Command Console software w4.2. Background signal correction was performed by applying the backgroundCorrect function from the limma package on the perfect match (PM) signals with R Software 3.3.1. The underlying model is the normal-exponential convolution model from RMA (chip intensity: addition of a signal exponentially distributed, chip noise: follows Gaussian distribution) [42]. The variance stabilizing transformation algorithm (justvsn function from the vsn package [43] was applied to the background corrected signal (monotonic transformation), and the signal then transformed back to its usual scale by exponentiation (base 2). To make the chips comparable, a quantile normalization [44] (normalize function from the affy package) was then applied to the variance-stabilized signal. The probe signals for replicated arrays were averaged and a quantile normalization performed anew. In all, 24,768 probes were analyzed.

Statistical analyses

Microbiome samples were clustered according to their genus composition by a nonmetric multidimensional scaling (NMDS) approach based on ecological distance matrices calculated by Bray-Curtis dissimilarities, as implemented in R (Vegan, metaMDS, and ggplot2 packages). NMDS ellipses were drawn based on a confidence interval (CI) of 0.95. To determine significant factors that describe the community structure better, we used a multivariate ADONIS test with terms added sequentially. The associations between baseline genus abundance or genus diversity, blood gene expression, and MVA-Nab response were evaluated by using the Spearman rank correlation test with significance defined by P-value < 0.05. The heatmap was performed with values row-centered and scaled, Pearson correlation as the distance method and a dendrogram computed and reordered based on row means. The heatmap, logistic regression analyses, and ROC curves were performed and generated with R. Ingenuity® pathway analysis (IPA®) was used to perform functional enrichment analyses and identify new targets or candidate biomarkers within the context of biological systems. It provided the canonical pathways, molecular/cellular functions, and networks that were statistically overrepresented in the gene signatures.

Ethics and community involvement

The study was conducted in accordance with the Declaration of Helsinki and the International Conference on Harmonization Good Clinical Practice guidelines and approved by the relevant regulatory and independent ethics committees. Each participant provided written informed consent before study entry. The study was registered and approved by the Peru regulatory authorities (IMPACTA IRB 0037-2014-CE; Peru NIH 396-2014-OG-OGITT-OPE/INS).

Results

Study of host microbiota before vaccination and relation to post-vaccination humoral responses

The study included five men and five women (18–45 years old) vaccinated by the intramuscular route to assess the safety and immunogenicity of MVA-HIV clade B (MVA-B), results reported elsewhere *(*Sanchez & Goncalves, manuscript submitted*).* Exploratory analysis of whole blood samples at two distinct time-points before vaccination (w-2 and w0) studied the gene expression profile and the skin and stool samples for microbiome analysis (w0) at baseline. As expected, the microbial composition differed between the skin and stool samples (**Supplemental Fig. 2a-c**). In addition, the stool samples showed dissimilarities between men and women, but this comparison did not reach statistically significant differences ($P < 0.097$) (**Supplemental Fig. 2a**). The predominant microbial families relatively abundant in skin samples were Moraxellaceae, Staphylococcaceae and Pseudomonadaceae, whereas Ruminococcaceae, Lachnospiraceae, Prevotellaceae, and Bacteroidaceae were predominant in stool samples (**Supplemental Fig. 2c**). The 16S RNA sequencing generated several metrics: richness (sobs: number of observed OTUs; chao: Chao1 richness estimate; ace: Abundance-based coverage estimation) and diversity (Shannon: Shannon diversity index; sd_invsimpson: inverse Simpson diversity index). The amplitude of the humoral response was defined by the MVA-specific IgG neutralizing antibodies measured in serum at w8 post-vaccination. We observed no correlation between the MVA-Nab response and the baseline indexes of diversity and richness in either skin or stool (data not shown). We did however find significant positive correlations between the abundance of both skin *Prevotella* ($r = 0.76$, $P = 0.0159$) (Fig. 1a) and fecal *Eubacterium* ($r = 0.68$, $P = 0.0351$) (Fig. 1b) at baseline with MVA-Nab response.

Whole blood gene expression and host microbiota before vaccination are associated with post-vaccination humoral responses

To improve our understanding of host molecular mechanisms potentially associated with skin and gut microbiota that may be involved in vaccine immunogenicity, we counted the number of genes at baselines that were correlated with the MVA-Nab response at w8. We confirmed that gene expression of the baseline samples did not differ between w-2 and w0 using hierarchal clustering analysis (**data not**

shown). Out of all samples, we found 154 significant genes correlated with the MVA-Nab response ($P < 0.05$) (Fig. 2a). However, no correlation was observed between genus diversity and MVA-Nab response.

Next, we looked for a correlation between the microbiota diversity index and the genes (n = 154) correlated at baseline with MVA-Nab responses. We found 22 genes for skin and 19 for stool that were correlated with at least one diversity index (Shannon or sd_invsimpson) (Fig. 2a, **Supplemental Tables 1 and 2**), including 10 common genes to the skin and stool samples. Among these genes, we observed one gene cluster positively correlated with MVA-Nab response and another negatively correlated with humoral response (Fig. 2a). According to the IPA analysis, the negatively correlated genes appear to be involved in protein transmembrane transport, translation and transcription regulation, cell division, migration, proliferation, and differentiation, as well as in the oxidation reduction and metabolic processes. The positively correlated genes, on the other hand, appeared involved in cell homeostasis and migration, cell growth, proliferation, regulation of gene expression, the apoptotic process, exocytosis, and intracellular signal transduction. Interestingly, among the 10 common genes to the skin and stool samples we found the IGLV8-61, BLK, and EBF1 genes which are involved in antigen recognition, B cell development, proliferation, and differentiation, and in the positive regulation of transcription in B cell and B cell receptor signaling (Fig. 2a). Surprisingly these three significant genes involved in B cell development stages were negatively correlated with the baseline abundance of *Prevotella* and *Eubacterium*, respectively for skin and stool (Fig. 2b). To assess the predictive power of this signature of three genes and each of the two microbial genera, we ran logistic regression models (Fig. 2c). Use of the expression of the three genes and *Prevotella* abundance in the skin microbiota has an 85.42% chance, assessed by its area under the curve, of correctly predicting MVA-Nab responders, while with the three-gene signature and *Eubacterium* abundance in the stool microbiota there is an 89.58% chance of correctly predicting MVA-Nab responders (Fig. 2c). These results suggest that advanced B lymphocyte differentiation before vaccination, potentially signaled by high expression of these three genes, and associated with low abundance of *Prevotella* or *Eubacterium*, is associated with poor MVA-Nab response.

Discussion

To our knowledge, this work is the first to investigate the crosstalk between pre-vaccination host gene expression in blood cells, skin and stool microbiota and their association with the intensity of ensuing post-vaccination Nab responses. The data may provide important guidance for future design and refinement of vaccine strategies aiming at the induction of neutralizing antibody-mediated immunity. The limitation of this study is the small number of individuals included. However, the strength of our work is the availability of two sets of gene expression data collected at baseline (w-2, w0) that is often absent in other studies. It is intriguing to discover three genes, all involved in B cell differentiation and proliferation correlated with humoral responses 2 months later. Further validation studies are necessary in the future.

First, we observed that the abundance of particular skin or stool bacteria were associated with the MVA-Nab response. Abundant *Prevotella* in the skin at baseline was positively correlated with MVA-Nab response. *Prevotella* is known to promote mucosal inflammation and to stimulate production of epithelial

cell cytokines [45]. *Prevotella* is also found in larger numbers in the skin of women aged 60–76 years than in that of women in their 20 s and 30 s and was enriched in all of the skin sites of the older group compared to the younger ones [46]. In stool, we found that *Eubacterium* abundance at baseline was positively correlated with the MVA-Nab response. This family of bacteria is known to be associated with gut health [47–49], and several of its species are higher in centenarians than in either young or elderly adults [50]. The potential impact of the gut microbiota on vaccine immunogenicity has been already investigated with systemic vaccines [51] and with oral vaccines including those of rotavirus (RVV), polio, and cholera, mainly in infants/children living in low-income countries [52, 53]. For example, bacteria related to *Streptococcus bovis* species were more abundant before vaccination in Ghanaian vaccine-responders than non-responders and were positively associated with RVV efficacy, whereas *Bacteroides* and *Prevotella* species were more common in the microbiome of nonresponders and correlated with a lack of RVV response [54]. In Bangladeshi infants, the pre-vaccination presence of *Bifidobacterium* was positively associated with some adaptive immunological responses, such as $CD4^+$ and $CD8^+$ T-cell proliferative responses to BCG and tetanus toxoid vaccinations as well as specific IgG responses to tetanus toxoid and hepatitis B vaccines, whereas high levels of enteric pathogens such as Enterobacteriales and Pseudomonadales were associated with neutrophilia and poorer vaccine responses [51].

Secondly, we examined the pre-vaccination host blood genes that were correlated with MVA-Nab intensity. We then investigated microbiota abundance to decipher a minimal gene signature predictive of MVA-Nab responsiveness. Interestingly, within this signature we find BLK, IGLV8-61 and EBF1 involved in B cell development, proliferation and differentiation and in the positive regulation of transcription in B cells and B cell receptor signaling. The BLK gene belongs to the family of protein tyrosine kinases src, and the B cells activation induces BLK gene product phosphorylation playing a key role in transmitting signals through surface immunoglobulins which supports the pro-B to pre-B transition and the signaling for growth arrest and apoptosis downstream of B-cell receptor [55]. BLK also plays a role in the development, differentiation, and activation of B cells and in the intracellular signaling pathway. BLK is detected in pro-B cells and persists in mature B cells but is absent in plasma cells. Triple protein tyrosine kinase (SFK)-deficient mice — BLK, LYN, and FYN — have impaired NFkB signaling and B cell development [56]. EBF1, an early B cell factor 1, is one of the transcription factors essential for orchestrating the development of the B cell line. Heterozygosity of EBF1 results in the deregulation of at least eight transcription factors involved in lymphopoiesis and the deregulation of key proteins that play a crucial role in the survival, development, and differentiation of pro-B cells [57]. IGLV8 (variable domain) is a glycoprotein produced by B lymphocytes; its binding of a specific antigen triggers the clonal expansion and differentiation of B lymphocytes into immunoglobulin-secreting plasma cells. The link between microbiota and host blood transcriptome has also been studied previously by Nakaya et al., who showed that TLR5 expression in blood 3 days after influenza vaccination was correlated with antibody response 28 days later [28]. This correlation was significantly lower in TLR5-deficient mice immunized with TIV compared to wild-type mice. As influenza vaccine does not stimulate TLR5 directly, however, Oh et al. demonstrated with germ-

free or antibiotic-treatment that the commensal bacteria were the source of the TLR5 ligands responsible for enhancing immune response to TIV [7].

Conclusion

It should be noted that in our study the three genes were negatively correlated with MVA-Nab response and microbial diversity of both skin and stool samples but also with the abundance of the *Prevotella* family in skin and the *Eubacterium* family in stool. The logistic regression based on the expression of these three genes and *Prevotella* and *Eubacterium* abundance for, respectively, skin and stool, highlights the predictive power of this signature for the MVA-Nab immune responses. These results propose that an advanced differentiation state of B lymphocytes before vaccination, potentially represented by a high expression of these three genes and associated with low genus abundance and diversity, might be associated with poor MVA-Nab response.

Declarations

Ethics approval and consent to participate

The study was conducted in accordance with the Declaration of Helsinki and the International Conference on Harmonization Good Clinical Practice guidelines and approved by the relevant regulatory and independent ethics committees. Each participant provided written informed consent before study entry. The study was registered and approved by the Peru regulatory authorities (IMPACTA IRB 0037-2014-CE; Peru NIH 396-2014-OG-OGITT-OPE/INS).

Consent for publication

Not applicable

Availability of data and materials

Raw Illumina MiSeq sequences and study metadata were deposited in the National Center for Biotechnology Information - NCBI repository (Bioproject accession number: XXXXXXXX, SRA accession number: XXXXXX).

Competing interest

CB is co-founder, shareholder and employee of Aelix Therapeutics, outside of this work.

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Author contribution

Conceptualization (BC, CB), Methodology (BC, CB, JS, RP), Funding (BC, CB, JS, RP), Acquisition and validation (EG, YG), Formal Analysis (EG, YG, BC), Investigation (EG, YG, BC, RP), Supervision (BC, CB, JS, JL, RP), Resources (BC, CB, JS, JL, RP), Data curation (EG, YG) - Writing (EG, YG, BC) - Original Draft preparation Writing (EG, YG) – Review Editing (all authors), Visualization (EG, YG), Project Administration (BC, CB, JS, RP).

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Figures

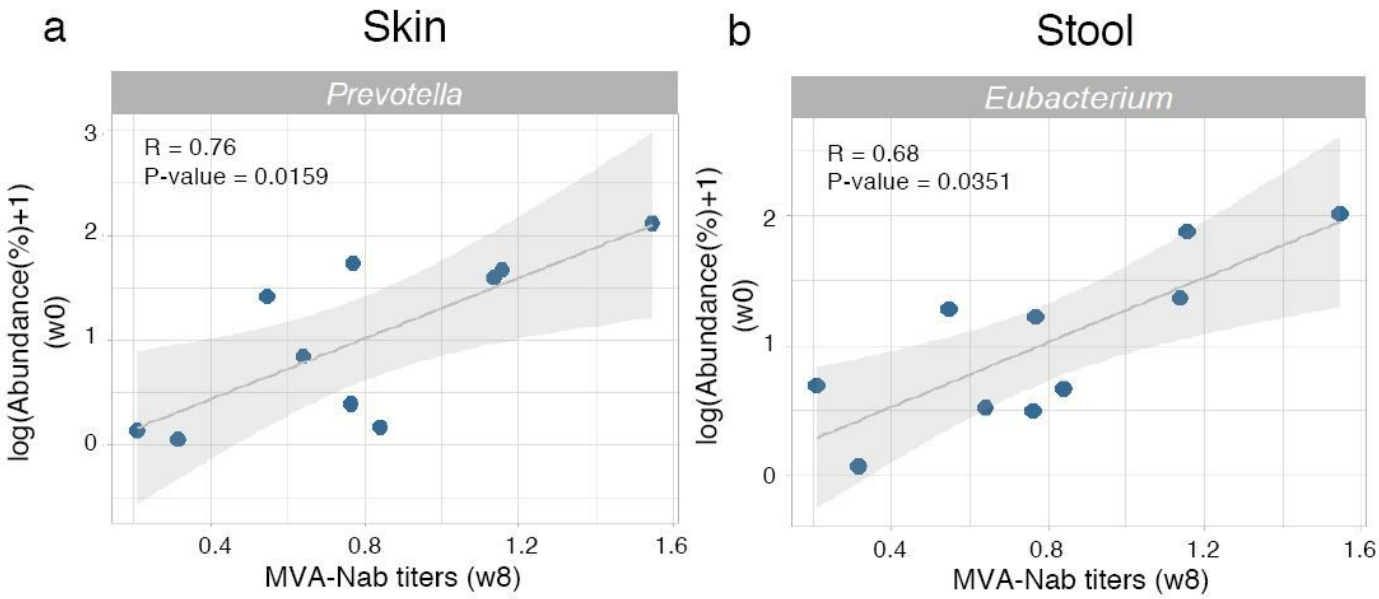
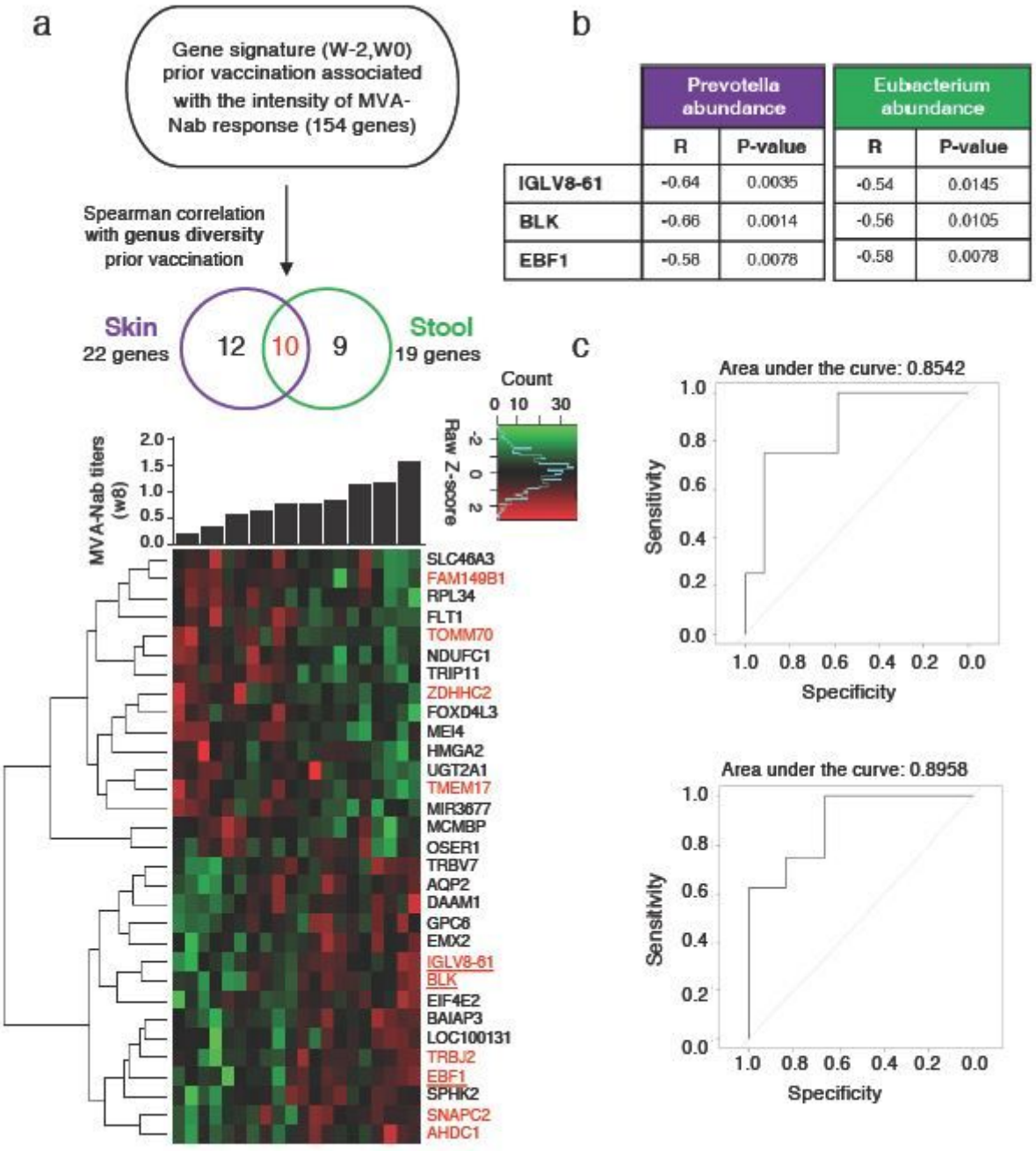


Figure 1

Microbial abundance before vaccination is correlated with MVA-Nab responses (a) Abundance of Prevotella in skin and (b) abundance of Eubacterium in stool are correlated with MVA-specific neutralizing antibody titers at w8 (log1/EC50). Spearman rank sum test was applied with a P-value < 0.05. All genera were filtered by a minimum median abundance of 0.1% across the samples.



	Prevotella abundance		Eubacterium abundance	
	R	P-value	R	P-value
IGLV8-61	-0.64	0.0035	-0.54	0.0145
BLK	-0.66	0.0014	-0.56	0.0105
EBF1	-0.58	0.0078	-0.58	0.0078

Figure 2

Blood gene expression combined with host microbiota before vaccination shapes MVA-B responses (a) Investigation of the blood gene expression (w-2, w0) correlated with MVA-Nab response (w8) and host genus diversity (w0), for skin (green) and stool (purple). The Spearman correlation test was applied with $P<0.05$ defined as statistically significant. The heatmap shows the expression profile of the 22 (purple) and 19 (green) genes correlated respectively with skin and stool, according to MVA-Nab response intensity from lowest to highest responders. The 10 common genes are colored in red. The color-gradient from green (-2, low) to red (2, high) indicates the intensity of gene expression. Among the genes

correlated with both skin and stool, three genes are underlined because they are involved in B cell functions. (b) Table shows the significant correlation coefficients and P-values for each of the three genes with the abundance of the genus correlated with MVA-Nab response. (c) ROC curves show the specificity and the sensitivity of the logistic regression models, i.e., the proportion of correctly predicted responders and nonresponders, respectively. The logistic regression is based on the expression of the minimal gene signature (IGLV8, EBF1, and BLK) and the abundance of Eubacterium and Prevotella, respectively, in stool and skin.

Supplementary Files

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- TableGenesFunctions.pdf
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