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Transcriptomic profiling of different responder types in adults after a Priorix® vaccination

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BO conceived the study; BO, PM, KL, AS, GM, HJ, PVD, PB, VVT supervised the experiments; EB, NDN, BO, PM, KL, AS, GM, PVD, PB, VVT designed the experiments; EB, NDN, PM, GE, SVDH performed the experiments; EB, NDN, PM performed the data-analysis; EB, NDN, PM interpreted the results; EB wrote the first draft of the paper. All authors contributed to the final draft. All authors attest they meet the ICMJE criteria for authorship.

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Abstract

Thanks to the recommendation of a combined Measles/Mumps/Rubella (MMR) vaccine, like Priorix®, these childhood diseases are less common now. This is beneficial to limit the spread of these diseases and work towards their elimination. However, the measles, mumps and rubella antibody titers show a large variability in short- and long-term immunity. The recent outbreaks worldwide of measles and mumps and previous studies, which mostly focused on only one of the three virus responses, illustrate that there is a clear need for better understanding the immune responses after vaccination. Our healthy cohort was already primed with the MMR antigens in their childhood. In this study, the adult volunteers received one Priorix® vaccine dose at day 0. First, we defined 4 different groups of responders, based on their antibody titers' evolution over 4 time points (Day 0, 21, 150 and 365). This showed a high variability within and between individuals. Second, we determined transcriptome profiles using 3'mRNA sequencing at day 0, 3 and 7. Using two analytical approaches, "one response group per time point" and "a time comparison per response group", we correlated the short-term gene expression profiles to the different response groups. In general, the list of differentially expressed genes is limited, however, most of them are clearly immune-related and upregulated at day 3 and 7, compared to the baseline day 0. Depending on the specific response group there are overlapping signatures for two of the three viruses. Antibody titers and transcriptomics data showed that an additional Priorix vaccination does not facilitate an equal immune response against the 3 viruses or among different vaccine recipients.

Introduction

Measles, mumps and rubella (MMR) used to be common childhood diseases. Thanks to the current 2-dose vaccination schedule for all children (In Belgium: first dose at 12 months and second dose between 11 and 12 years [1]), both recommended by the WHO (World Health Organization) and most National Immunization Technical Advisory Group (NITAGs), the incidence of MMR cases has decreased with 99% to on average only a few hundred cases reported per year in Belgium. Immunization against MMR is currently combined in one vaccine (for example Priorix® or MMR VAX PRO®), containing three live-attenuated virus strains. This vaccine type is considered to induce the strongest possible immune response without causing disease. Nevertheless, the short- and long-term immune responses after MMR vaccination are highly variable and inconsistent over time. A single dose of the MMR vaccine has been shown to be 93% effective against measles, 78% effective against mumps, and 97% effective against rubella (CDC) [2]. Following the CDC-recommended childhood vaccination schedule, two doses of the MMR vaccine are at least 97% effective against measles and 88% effective against mumps (rubella numbers not available, CDC) [2]. Children who underwent and survived a natural infection are considered to be permanently protected for life. However, individuals who have never been vaccinated, who remain non-responders after both doses or who were never exposed to the natural infection in their childhood, are at risk for infection and complications at a later age. These infections during adulthood are known to have a higher severity. Therefore, MMR vaccination is still recommended for adults at high risk: a second dose for adults who only received one primary dose or a full 2-dose vaccination for unvaccinated individuals (https://www.cdc.gov/vaccines/vpd/mmr/hcp/recommendations.html) (Belgian NITAG). Offering a second dose at any age can also help to limit the spread of MMR infections. Nowadays, 88% of measles, mumps or rubella outbreaks are due to unvaccinated individuals (CDC) [2]. The remaining 12% are due to vaccine non-responders or individuals that have lost their protective immunity over time [3,4].

Given the recurring outbreaks in recent years [5,6] and the fact that previous studies mostly focused on only one of the three virus responses [3,7–16], there is a need to assess the complete transcriptome profile in recipients of the MMR vaccine. The combination of this profile with the three separate MMR antibody titers could give us more insights in the variation of the immune response after MMR vaccination and may eventually result in a guideline for a new vaccination strategy.

In this MMR vaccination study, we correlate short term gene expression profiles of 40 healthy individuals, before (day 0) and after vaccination (day 3 and 7), with the corresponding evolution of the humoral immunity against measles, mumps and rubella, measured by the three separate antibody titers at day 0, 21, 150 and 365. Measuring the effect of a vaccination on the gene expression level can teach us how the immune system responds to three different viruses at the same time.

Material and methods

Study cohort

Forty healthy individuals (between 20 and 30 years) whom have received at least one MMR vaccine dose earlier in life, were recruited for this study (Supplementary table 1: data previous vaccinations). These individuals were administered one vaccination dose of Priorix® (a live attenuated MMR vaccine). Before vaccination (day 0) and at days 3, 7, 21, 150 and 365, blood samples were collected from each individual. At days 0, 3 and 7, blood samples were collected in two PaxGene blood RNA tubes (PreAnalytiX GmbH). These tubes contain a buffer that stabilizes the *in vivo* gene expression profile by minimalizing *in vitro* RNA degradation. At days 0, 21, 150 and 365, serum samples were collected and immediately stored at -80°C. The serum samples were sent in batch to the Antwerp University Hospital. The antibody (Ab) titers for measles, mumps and rubella were measured by the LIAISON® using the chemiluminescence immunoassay (CLIA) technology. Figure 1 shows the detailed timeline.

RNA extraction

RNA extraction from blood collected in 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 a fragment analyzer (Advanced Analytical, DNF-471 Standard Sensitivity RNA Analysis Kit). No RNA samples had to be excluded based on low quality.

3'mRNA sequencing

The experimental set-up is similar to the workflow reported by Bartholomeus et al., 2018. In brief, all RNA samples were prepared with the QuantSeq 3'mRNASeq Library Prep Kit FWD for Illumina (Lexogen GmbH) following the standard protocol for long fragments. The 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).

NGS data processing

Raw data from the NextSeq was demultiplexed and further processed through an in-house developed 3'mRNA sequencing pipeline. The quality of all reads was evaluated using FastQC (v0.11.5) before and after processing with Trimmomatic (v0.36). Trimmomatic was used to remove the leading 20 bases from reads, ensure a minimum quality score of 15 over a sliding window of 4 bases and require a minimum read length of 30 bases. Usage of oligodT primers could cause poly-A stretches at the 3' end. To remove these poly-A stretches, the 3' read end was trimmed with our own in-house poly-A removal script. All remaining sequences were mapped against the human reference genome build 38 (polymorph variants excluded) with HISAT2 (v2.0.4). HTseq (v0.6.1) was used to count all reads for each gene and set up a read count table.

Differential gene expression analysis and gene ontology enrichment analysis

Differential gene expression analysis was performed using the DESeq2 Bioconductor package [17]. For any given differential gene expression analysis, genes were removed prior to the analysis if they had

less than 200 read counts (5 times the number of individuals included in the study) over all samples considered during the analysis. Gene ontology enrichment analysis was performed on significantly differentially expressed genes using GOA tools [18]. To determine significantly enriched/depleted gene ontology terms related to biological processes, a Fisher's exact test was performed with Benjamini-Hochberg correction for multiple testing (FDR < 0.05). As a reference background set of genes, we used all genes (total number: 18180) measured during the 3'-mRNAseq experiment.

All codes used for the preprocessing and analysis of the data within this manuscript as well as the codes used to generate results and the DESeq2 results itself, are publicly available on github at https://github.com/NDeNeuter/mmr_rnaseq.

Ethical approval

This non-commercial investigator-driven study was approved by the medical ethical committee of the Antwerp University Hospital/Antwerp University, Belgium (EC15_19_210). All participants signed an informed consent form.

Results

Response groups

With the MMR study vaccine, the three live-attenuated MMR viruses were administered simultaneously at day 0, leading to one "combined" immune response. The humoral immune response against the vaccine were determined by measuring three separate antibody titers: anti-Measles IgG (AU/mL), anti-Mumps IgG (AU/mL) and anti-Rubella IgG (IU/ml). For classification of the immune responses, a hierarchical clustering method was applied on the antibody (Ab) titers at day 0 (pre-vaccination baseline) and days 21, 150 and 365 (post-vaccination). This clustering technique groups individuals with similar patterns in Ab titer evolution. This avoids using cut-off values, which may be biased. As shown in Figure 2, four different response groups were identified for each antibody titer: (a) High Ab: individuals with a relatively high Ab titer before vaccination that remained stable or further

increased after vaccination (b) Low Ab: individuals with a relatively low Ab titer before vaccination that remained low after vaccination (c) Long response: individuals with a relatively low Ab titer before vaccination that increased after vaccination and stayed stable within the first year (d) Peak response: individuals with a relatively low Ab titer before vaccination that increased at day 21, and then decreased by day 150 and 365. Table 1 shows the antibody titer response for each individual. The humoral immune response to each of the three attenuated vaccine viruses clearly varies between and within individuals, as shown earlier [19,20]. For example, individual 8 is a high Ab responder for measles, a long responder for mumps and a low Ab responder for rubella.

For both measles and mumps, 12/40 (30%) of the individuals had a high Ab titer at day 0 (before vaccination). In addition, 17/40 (42.5%) and 21/40 (52.5%) were able to gain high Ab titers for measles and mumps respectively without a loss of protection within the first year (long response). In contrast, the high Ab group for rubella contained only 3 individuals, whereas the majority of individuals (23/40) were classified in the low Ab group. While the remaining 35% (14/40) of the individuals had a peak response for rubella, measured at day 21, they were not able to sustain these higher anti-rubella IgG levels, which had declined by day 150 and 365. The peak response profile was thus mainly observed within the rubella Ab titer measurements.

Short term gene expression profiling

Since the three live-attenuated viruses are administered in a single vaccine, gene expression to each virus cannot be measured independently. However, gene expression changes can be determined in relation to the three different Ab titers at a given time point for each individual. We therefore performed two different analyses, one where the gene expression for a particular response group was compared to the other response groups and this for each Ab (virus) at a given time point (response group per time point) and one where the gene expression within a particular response group was compared at different time points and this for each Ab (virus) (time comparison per response group). These analyses resulted in a list of differentially expressed genes (DEGs) with a positive or negative

log2 fold change and an adjusted p-value, which can be related to biological processes by testing for gene ontology enrichment.

Response group per time point analysis

In this first (vertical) approach, one response group is selected for a specific Ab (virus) at a single time point. The gene expression profile of that response group is compared to the profile of the remaining groups for that specific virus at the same time point. For example, one analysis compared the expressed genes within the high Ab response group of measles at day 0 with the expressed genes of the remaining cohort of measles (low Ab, long response and peak response) at day 0. Results are shown in Table 2. Overall, this response group per time point analysis results in a very limited list of DEGs, with only a few immune-related genes (See supplementals, Supplementary table 2).

For measles, the high Ab group did not show any differential expression compared to the 3 other Ab groups at any time point. In the low Ab group four interferon (IFN) inducible genes were upregulated at day 0 before vaccination, compared to the 3 other Ab groups. These four IFN-inducible genes contribute to type I IFN responses and signaling and are related to functional anti-viral GO terms. Comparisons with the measles low Ab group showed no DEGs at day 3 and 7 at all. The measles long response and the peak response comparisons both featured no immune-related DEGs at day 0 and 3. However at day 7, alpha defensins (DEFA1/1B/3) were downregulated in the long response group and (DEFA1/1B/3/4) upregulated in the peak response group. These defensins are antimicrobial and cytotoxic peptides, involved in host defense mechanism, which are highly abundant in the granules of neutrophils, the largest group of granulocytes. Further, IFITM3, another IFN-inducible protein with antiviral capacities, is also significantly downregulated in the measles peak response group compared to the other groups.

For mumps, no immune-related DEGs were found in comparison with the high Ab group. In the low Ab group, similarly as for measles, the IFN-inducible gene, MX1, was upregulated at day 0. Also, CASP5, coding for a caspase in the lectin complement cascade, is upregulated in the mumps low Ab group at

day 0. Furthermore, 5 genes were downregulated in the low Ab group compared to the other Ab groups at day 7. Those 5 genes contribute to general defense response (including IFN), NK cells chemotaxis and cytotoxicity and granzyme-mediated apoptosis GO terms. The mumps long response group at day 7 showed downregulation for 3 genes, including MX1. SIGLEC1 is a lectin-like adhesion molecule that binds different lymphocytes and is considered as member of the immunoglobin (Ig) family. FCGR2B is part of the Fc receptor of IgG and contributes to different B-cell GO terms.

For rubella, the high Ab group, consisting of only 3 individuals, showed immune-related DEGs at each time point: upregulation of CD40 at day 0 (involved in B-cell isotype switching); upregulation of XCL1 at day 3 (chemotactic activity towards lymphocytes); upregulation of IGH (the heavy locus of IgG) and downregulation of both HLA-DRB5 and APOBEC3A (an anti-viral DNA deaminase) at day 7. The rubella low Ab group, which is the largest group, had no significant DEGs related to the immune system. The rubella peak response comparison showed downregulation of IFITM3 at day 3.

Time comparison per response group analysis

In this second (horizontal) approach only individuals in a particular response group per Ab (virus) are considered. This analysis compared the gene expression profile at a post-vaccination time point to the baseline profile (Day 0 – before administration of the vaccine). The results are shown in Table 3 (Supplementary table 2: functions).

For measles, the high Ab group showed no significant differences at day 3 and 7, compared to day 0. In the low Ab group, only the day 7 comparison revealed three upregulated genes related to immunity: UBB (Ubiquitin B), IFITM3, and ACKR1 (atypical chemokine receptor 1). In contrast, several DEGs were observed in the longitudinal comparison of the measles long response group. The day 3 vs day 0 comparison revealed upregulated genes related to interleukin regulation, Toll-like signaling and IFN related terms. The day 7 vs day 0 comparison showed additional upregulation of genes involved in IFN and cytokine signaling pathways, cellular responses and Ab processing/presentation, viral transcription and replication processes, and T-cell signaling. Time point comparison of the measles peak response

group only showed 2 upregulated genes for the day 3 vs day 0 comparison: IFI6 (IFN inducible protein) and TUSC2.

For mumps, in the high Ab group only upregulated genes could be found between day 7 and day 0. These 11 genes are related to viral processes, IFN and cytokine mediated signaling GO terms. The mumps low Ab group longitudinal comparison only showed a downregulation of PRTN3, which is related to neutrophil extravasation. Similar to what was observed for measles, the long response group showed the most immune-related DEGs at both post-vaccination time points. These DEGs contained mostly defensins and were all consistently upregulated at the later time point. Gene ontology analysis reveals the following upregulated processes for the mumps long response group after vaccination: Innate responses incl. cytokines, IFN, ILs and TNF, immune effector responses, viral transcription/replication and neutrophil activation and degranulation.

For rubella, the high Ab group showed a similar pattern to that observed for mumps high Ab group: immune-related DEGs are found only between day 7 and day 0, all upregulated, except for IRF3. Gene ontology analysis reveals enrichment for cellular response to virus, different viral processes and toll-like receptor signaling among these genes. The rubella low Ab group on the other hand was more comparable with the measles low Ab group: two upregulated DEGs at day 7 vs 0, including ACKR1 (related to chemokine/cytokine binding) and DEFA4 (part of innate immune system). In the rubella peak response group, 10 upregulated immune-related DEGs were observed between day 7 and day 0. Those 10 DEGs featured the following enriched GO processes: innate and humoral immune response, cytokine stimulus and inflammatory response and neutrophil activation and degranulation.

Discussion

Live-attenuated virus vaccines are known to trigger a strong immune response, as they are most similar to a natural infection. As the MMR vaccine is known to generate a robust cell-mediated immune response, it should result in a sustainable protection level. However, our results indicate that

vaccination with the 3 different live-attenuated MMR viruses does not result in a consistent and shared immune response against the three viruses [19–23]. Indeed each one of the vaccinees showed a different response profile for measles, mumps and rubella (Table 1). These results correspond well with the reported variability of the MMR vaccine response [2]. However, there are two aspects to be considered here. First, this was an additional dose of the vaccine given to adults, who had already received one or two doses during childhood (Supplementary table 1). There is thus a large baseline variability present between and within individuals caused by the previous MMR vaccination. The acquired immunity from the previous vaccine doses could have declined at a different rate for each of the three viruses. Second, there are no clinical thresholds set for MMR Ab titers to determine non-, low and strong responders. To handle the differences in variability, we used a formal clustering strategy to define 4 different types of response based on the evolution of each Ab titer over 1 year post-vaccination. This led to non-uniform group sizes, where the measles peak response and rubella high response group only had 4 and 3 individuals, respectively. The observation of these different antibody titer profiles captured by these groups suggest that there is a large individual variability in the immune response against the different viral components. Most of the cohort only showed increased antibody titers for one or at most two components post-vaccination. Long-term increases could be observed mainly for either measles or mumps, while in 92.5% of the cohort, the increase of the rubella Ab titer was of short duration (low Ab and peak response group). The exact cause of this inability to build up consistent high Ab protection levels against all 3 MMR viruses could not be defined based on the gene expression profiles. The difference in immunogenicity for each vaccine component within monovalent vaccines compared to combined vaccines is less studied for MMR [24–27]. As monovalent vaccines require more shots and create a delay in protection for all three infections due to separate vaccination schedules, health care providers and pharma industry are focused on developing combined vaccines [28]. For example, the production of ATTENUVAX® (Measles Virus Vaccine Live), MUMPSVAX® (Mumps Virus Vaccine Live), and MERUVAX®II (Rubella Virus Vaccine Live) was shut down in 2009 (Merck).

285 Prior studies of the MMR vaccines have mostly been based on the serological responses [3,8,29].
286 Rager-Zisman et al. added also flow cytometry data with a focus on CD4/CD8 lymphocyte ratio and NK
287 cells activity [30]. Poland et al., studied the MMR vaccine extensively, but mostly focused on only one
288 of the 3 viral components: cytokine data [12,16], measles specific or rubella specific humoral immunity
289 with proteome microarrays [11,15] and HLA typing [13–15]. Transcriptomic analyses have been
290 performed on PBMCs of previously vaccinated highest responders and lowest non-responders after *in*
291 *vitro* measles virus stimulation [10], or with the live rubella virus [7]. These studies found that genes
292 related to plasma cell survival and CD93 were associated with measles-specific antibody response after
293 vaccination, which corresponds with the gene expression difference found in the study. Further,
294 antigen presentation genes and inflammation-related genes were postulated in these studies to
295 explain rubella vaccine-induced immune response variations. There is a certain overlap of GO
296 enrichments with our study results. However, these studies focused on one virus response at a time in
297 preselected MMR responders using a cell culture reactivation set-up with only one of the three virus
298 strains [7,10,29]. Thus the trivalent combined immune response measured in this study cannot be
299 directly compared to these single immune response.

300 The seroconversion against the 3 viruses in children after vaccination has been reported to be more
301 straightforward [12,30]. Our cohort of healthy individuals was unintentionally limited in age
302 categories. All participating individuals were born between 1987 and 1997, after the start of the MMR
303 vaccination program in Belgium. The cohort thus contained individuals between 20 and 30 years old
304 when the study took place in 2017. Due to this young cohort, we did not expect any age-related
305 immune system and health issues. Half of the volunteers completed the two dose vaccination schedule
306 [1], based on reporting and childhood records. The other 50% of the cohort reported at least one MMR
307 vaccination during their childhood (12 months) or at adolescence (10-12 years). The full overview of
308 previous MMR vaccination history for our cohort is provided in Supplementary material 1. However,
309 the MMR vaccination history (1 or 2 doses) does not seem to have an impact on the response types of
310 this study vaccination (Pearson's Chi-squared test, p= 0.8136). It is notable that there is seemingly no

relationship between those that have completed the two dose vaccination schedule and those that were categorized as High Ab by clustering. This result is unexpected as this schedule is currently deemed as sufficient for long-term protection in most individuals. Indeed, this lack of association may be due to the small sample size of this study, the age limits of the study cohort, incomplete vaccination records/recall or the biased clustering approaches used to define the High Ab group. What also could lead to such insignificance, is that volunteers who recall only one dose probably received a second dose as well, if we look to the age categories. On the other hand, recent outbreaks show that about 12% of the infected consisted of vaccinated individuals [5,6]. The low number of volunteers in the High Ab groups may suggest that there is a need to increase protection levels in the population should outbreaks continue. This could be accomplished by optimization of the vaccination schedule, also for adults, or to adapt the MMR vaccine to increase the long-term immunogenicity.

Few immune-related DEGs were found when comparing the different responder groups across different time points (Table 2). Twenty out of the 30 different comparisons showed no immune-related or no significant DEGs at all. Lack of DEGs may suggest that any gene expression changes were consistent across response groups for each specific viral component, or that any gene expression differences may have been not large enough to be picked up because of the small sample size. The gene expression profiles for rubella are the most divergent, as expected because of the large number of low rubella Ab titers at day 0, prior to vaccination. While it is important to not overly conjecture based on specific DEGs due to the known high false positive rate, two main observations from the overall cross-responder group analysis can be considered. Firstly, at day 0 (baseline), we found upregulated genes (IFN inducible genes/caspase 5) in the measles and mumps low Ab groups and an upregulated CD40 marker (TNF receptor on antigen presenting cells) in the rubella high Ab group. It was unexpected to find differences in the gene expression at baseline in healthy individuals prior to vaccination. However, our previous hepatitis B vaccine study also found significant immune related differences in non-responders at the day 0 baseline [31]. As such, baseline gene expression may play a larger role in determining vaccine outcomes than is currently appreciated. Secondly, while rubella is

a single-stranded RNA virus, we found a downregulated DNA cytidine deaminase, APOBEC3A, at day 7 in the rubella high Ab group. However, latest literatures suggest also a role in RNA editing and acting on the pathogen itself and their host RNA [32,33]. What the relation is between the live-attenuated rubella virus and the downregulation of APOBEC3A is unknown. And finally, it seems that alpha defensins are still expressed at the peak response: DEFA1, 3 and 4 are transcribed to cytotoxic proteins, stored in neutrophilic granules. Expression of these genes is upregulated in the peak response and downregulated in the long response. This could indicate that neutrophilic degranulation is still ongoing and/or the granules have to be refilled. A follow-up study could focus on the protein load of neutrophils.

Using day 0 as an uninfluenced immune baseline to determine significant changes in gene expression at day 3 and 7 resulted in a larger amount of DEGs, except for the high Ab measles group. The gene expression stability in the high Ab measles group could be the result of the high level of previously established immunity against the measles virus. All immune-related DEGs found for the other groups are upregulated both at day 3 and day 7 when compared to the baseline (day 0) situation, except for PRTN3 (Low Ab Mumps, 0 vs 3), related to neutrophil extravasation, and IRF3 (high Ab rubella, 0 vs 7), an IFN regulatory transcription factor. In general, all DEGs are known to be IFN-related or are known to play a role in the innate immune system, like the alpha defensins. This matches prior knowledge that a large part of the antiviral immune response might be IFN-based [34–36].

The complexity of the diverse responses to the different components of the vaccine, even within a single individual, makes a straightforward vaccine-related gene expression pattern not possible. Furthermore, this vaccination was an additional dose given to adults, following their childhood vaccinations. This means that the immune system has already been exposed to these viruses, and the elicited “secondary” immune response could be faster or lower than would be the case with a “de novo” vaccination. Comparatively however, there are far fewer or even no DEGs in all low Ab groups than found with the other response groups. This is expected as the low Ab groups are expected to have

a low immune response to the viral component before and after vaccination. Overall the day 3 vs 0 analysis tended to find less DEGs than the day 7 vs 0, but both remained small compared to overall gene expression changes occurring in healthy individuals on a daily basis. Furthermore, we only see significant DEGs associated with the innate immune system, but none related to the adaptive cell-mediated or the humoral immunity. Given the mechanisms of vaccination, one would expect these to be present. Thus these results indicate that an additional dose does not elicit a strong immunogenic response reflected by gene expression changes. The small numbers of DEGs could also suggest that the impact of the adaptive immune response on the gene expression was restricted to an earlier time point than was measured.

Conclusion

Antibody titers and gene expression levels varied greatly within and across individuals in MMR vaccine-induced immune responses against the 3 viruses. The largest difference in gene expression was observed for those individuals who had a significant change in antibody titers following vaccination, compared to smaller gene expression changes in those individuals that maintained consistent Ab titers (either low or high). However, any gene expression changes stayed limited in size and in time compared to other possible interventions, largely independent of the previously established immune protection. This indicates that the effect of additional MMR vaccinations on the immune system is very minor and that any measurable effects subside in a matter of days. As a consequence, our results highlight a need to further study the individual host's and public health benefits of extra doses at different stages of adult life. Such insights will become critical as the MMR vaccinated population gains in size and grows older.

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References

[1] European centre for disease prevention and control. Vaccine schedule 2019. https://vaccine-schedule.ecdc.europa.eu/.

[2] Center for Disease Control and prevention. Measles, Mumps, and Rubella (MMR) Vaccination: What Everyone Should Know 2019.

[3] Seagle EE, Bednarczyk RA, Hill T, Fiebelkorn AP, Hickman CJ, Icenogle JP, et al. Measles, mumps, and rubella antibody patterns of persistence and rate of decline following the second dose of the MMR vaccine. Vaccine 2018;36:818–26. https://doi.org/10.1016/j.vaccine.2017.12.075.

[4] Haralambieva IH, Ovsyannikova IG, Pankratz VS, Richard B. The genetic basis for interindividual immune response variation to measles vaccine: new understanding and new vaccine approaches 2013;12:57–70. https://doi.org/10.1586/erv.12.134.The.

[5] Czajka H, Czajka S, Dylag KA, Borek E, Kuchar E. Vaccination Against Measles, Mumps, and Rubella in the Light of Current Epidemic Threats: Unjustified Postponement. Adv Exp Med Biol 2019. https://doi.org/10.1007/5584_2019_344.

[6] Thornton J. Measles cases in Europe tripled from 2017 to 2018. BMJ 2019;364:l634. https://doi.org/10.1136/bmj.l634.

[7] Haralambieva IH, Oberg AL, Ovsyannikova IG, Kennedy RB, Grill DE, Middha S, et al. Genome-Wide Characterization of Transcriptional Patterns in High and Low Antibody Responders to Rubella Vaccination. PLoS One 2013;8. https://doi.org/10.1371/journal.pone.0062149.

[8] McLean HQ, Fiebelkorn AP, Ogee-Nwankwo A, Hao LJ, Coleman LA, Adebayo A, et al. Rubella virus neutralizing antibody response after a third dose of measles-mumps-rubella vaccine in young adults. Vaccine 2018;36:5732–7. https://doi.org/10.1016/j.vaccine.2018.08.010.

[9] Haralambieva IH, Gibson MJ, Kennedy RB, Ovsyannikova IG, Warner ND, Grill DE, et al. Characterization of rubella-specific humoral immunity following two doses of MMR vaccine using proteome microarray technology. PLoS One 2017;12:1–12. https://doi.org/10.1371/journal.pone.0188149.

[10] Haralambieva IH, Zimmermann MT, Ovsyannikova IG, Grill DE, Oberg AL, Kennedy RB, et al. Whole transcriptome profiling identifies CD93 and other plasma cell survival factor genes associated with measles-specific antibody response after vaccination. PLoS One 2016;11:1–14. https://doi.org/10.1371/journal.pone.0160970.

[11] Haralambieva IH, Simon WL, Kennedy RB, Ovsyannikova IG, Warner ND, Grill DE, et al. Profiling of measles-specific humoral immunity in individuals following two doses of MMR vaccine using proteome microarrays. Viruses 2015;7:1113–33. https://doi.org/10.3390/v7031113.

[12] Haralambieva IH, Ovsyannikova IG, O'Byrne M, Pankratz VS, Jocobson RM, Poland GA. A large observational study to concurrently assess persistence of measles specific B-cell and T-cell immunity in individuals following two doses of MMR vaccine. Vaccine 2013;29:4485–91. https://doi.org/10.1038/mp.2011.182.doi.

[13] Ovsyannikova IG, Jacobson RM, Ryan JE, Vierkant RA, Pankratz VS, Jacobsen SJ, et al. HLA class II alleles and measles virus-specific cytokine immune response following two doses of measles vaccine. Immunogenetics 2005;56:798–807. https://doi.org/10.1007/s00251-004-0756-0.

[14] Ovsyannikova IG, Jacobson RM, Vierkant RA, Pankratz VS, Poland GA. HLA supertypes and immune responses to measles-mumps-rubella viral vaccine: Findings and implications for vaccine design. Vaccine 2007;25:3090–100. https://doi.org/10.1016/j.vaccine.2007.01.020.

[15] Ovsyannikova IG, Schaid DJ, Larrabee BR, Haralambieva IH, Kennedy RB, Poland GA. A large population-based association study between HLA and KIR genotypes and measles vaccine

antibody responses. PLoS One 2017;12:1–18. https://doi.org/10.1371/journal.pone.0171261.

[16] Umlauf BJ, Haralambieva IH, Ovsyannikova IG, Kennedy RB, Pankratz VS, Jacobson RM, et al. Associations Between Demographic Variables and Multiple Measles-Specific Innate and Cell-Mediated Immune Responses After Measles Vaccination. Viral Immunol 2012;25:29–36. https://doi.org/10.1089/vim.2011.0051.

[17] Love MI, Huber W, Anders S. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. Genome Biol 2014;15:550. https://doi.org/10.1186/s13059-014-0550-8.

[18] Klopfenstein D V., Zhang L, Pedersen BS, Ramírez F, Vesztrocy AW, Naldi A, et al. GOATOOLS: A Python library for Gene Ontology analyses. Sci Rep 2018;8:1–17. https://doi.org/10.1038/s41598-018-28948-z.

[19] Vandermeulen C, Clement F, Roelants M, Van Damme P, Hoppenbrouwers K, Leroux-Roels G. Evaluation of Cellular Immunity to Mumps in Vaccinated Individuals with or without Circulating Antibodies up to 16 Years after Their Last Vaccination. J Infect Dis 2009;199:1457–60. https://doi.org/10.1086/598482.

[20] Vandermeulen C, Verhoye L, Vaidya S, Clement F, Brown KE, Hoppenbrouwers K, et al. Detection of mumps virus-specific memory B cells by transfer of peripheral blood mononuclear cells into immune-deficient mice. Immunology 2010;131:33–9. https://doi.org/10.1111/j.1365-2567.2010.03263.x.

[21] Vandermeulen C, Leroux-Roels G, Hoppenbrouwers K. Mumps outbreaks in high vaccinated populations: What makes good even better? Hum Vaccin 2009;5:494–6. https://doi.org/10.4161/hv.7943.

[22] Vandermeulen C, Roelants M, Vermoere M, Roseeuw K, Goubau P, Hoppenbrouwers K. Outbreak of mumps in a vaccinated child population: A question of vaccine failure? Vaccine

2004;22:2713–6. https://doi.org/10.1016/j.vaccine.2004.02.001.

[23] Vandermeulen C, Mathieu R, Geert LR, Pierre VD, Karel H. Long-term persistence of antibodies after one or two doses of MMR-vaccine. Vaccine 2007;25:6672–6. https://doi.org/10.1016/j.vaccine.2007.07.008.

[24] Mi K, Ou X, Guo L, Ye J, Wu J, Yi S, et al. Comparative analysis of the immunogenicity of monovalent and multivalent rotavirus immunogens. PLoS One 2017;12:1–15. https://doi.org/10.1371/journal.pone.0172156.

[25] Duval B, Gîlca V, Boulianne N, Deceuninck G, Rochette L, De Serres G. Immunogenicity of two paediatric doses of monovalent hepatitis B or combined hepatitis A and B vaccine in 8-10-year-old children. Vaccine 2005;23:4082–7. https://doi.org/10.1016/j.vaccine.2004.07.022.

[26] Overbosch D, Peyron F, Picot N, Varichon J-P, Dumas R, Chambonneau L, et al. Combined Typhoid Fever and Hepatitis A Vaccine: Comparison of Immunogenicity and Safety to Concomitant Monovalent Vaccine over 3 Years. J Travel Med 2006;12:319–26. https://doi.org/10.2310/7060.2005.12604.

[27] Kallinowski B, Knoll A, Lindner E, Sanger R, Stremmel W, Vollmar J, et al. Can monovalent hepatitis A and B vaccines be replaced by a combined hepatitis A/B vaccine during the primary immunization course? Vaccine 2001;19:16–22.

[28] Center for Disease Control and prevention. Monovalent vaccines—ATTENUVAX® (measles vaccine), MUMPSVAX® (mumps vaccine) and MERUVAX® (rubella vaccine) 2009.

[29] LeBaron CW, Beeler J, Sullivan BJ, Forghani B, Bi D, Beck C, et al. Persistence of measles antibodies after 2 doses of measles vaccine in a postelimination environment. Arch Pediatr Adolesc Med 2007;161:294–301. https://doi.org/http://dx.doi.org/10.1001/archpedi.161.3.294.

[30] Rager-Zisman B, Bazarsky E, Skibin A, Chamney S, Belmaker I, Shai I, et al. The effect of measles-mumps-rubella (MMR) immunization on the immune responses of previously immunized primary school children. Vaccine 2003;21:2580–8. https://doi.org/10.1016/S0264-410X(03)00053-7.

[31] Bartholomeus E, De Neuter N, Meysman P, Suls A, Keersmaekers N, Elias G, et al. Transcriptome profiling in blood before and after hepatitis B vaccination shows significant differences in gene expression between responders and non-responders. Vaccine 2018;36:6282–9. https://doi.org/10.1016/j.vaccine.2018.09.001.

[32] Sharma S, Patnaik SK, Thomas Taggart R, Kannisto ED, Enriquez SM, Gollnick P, et al. APOBEC3A cytidine deaminase induces RNA editing in monocytes and macrophages. Nat Commun 2015;6:6881. https://doi.org/10.1038/ncomms7881.

[33] Perelygina L, Chen M, Suppiah S, Adebayo A, Abernathy E, Dorsey M, et al. Infectious vaccine-derived rubella viruses emerge, persist, and evolve in cutaneous granulomas of children with primary immunodeficiencies. PLOS Pathog 2019;15:e1008080.

[34] Civas A, Island M, Génin P, Morin P, Navarro S. Regulation of virus-induced interferon-A genes. Biochemie 2002;84:643–54.

[35] Mesplède T, Navarro S, Génin P, Morin P, Island ML, Bonnefoy E, et al. Positive and negative control of virus-induced interferon-A gene expression. Autoimmunity 2003;36:447–55. https://doi.org/10.1080/08916930310001602119.

[36] Génin P, Vaccaro A, Civas A. The role of differential expression of human interferon-A genes in antiviral immunity. Cytokine Growth Factor Rev 2009;20:283–95. https://doi.org/10.1016/j.cytogfr.2009.07.005.

Figure legends

Figure 1: Overview of the MMR study time line.

Figure 2: Overview of all antibody titers. A hierarchical clustering method is used to define four response groups: High Ab, Low Ab, Peak response and long response.

Keywords: Priorix, Measles-mumps-rubella vaccination, RNA-sequencing, gene expression profiling,