Antiviral Research 166 (2019) 35–41

Contents lists available at ScienceDirect

Antiviral Research

journal homepage: www.elsevier.com/locate/antiviral

Genomic signature of early T-cell response is associated with lower antibody titer threshold for sterilizing immunity

Eugenia Z. Ong[a,b], Esther S. Gan[a,b], Ruklanthi de Alwis[a,b], Limin Wijaya[c], Xin Mei Ong[a,b], Menglan Zhang[d], Abigail WL. Wong[c], Yin Bun Cheung[e,f], Raphaël M. Zellweger[a,b], Eng Eong Ooi[a,b,g,h], Jenny G. Low[a,b,c,*]

[a] Viral Research and Experimental Medicine Centre @ SingHealth-Duke NUS, Singapore
[b] Programme in Emerging Infectious Diseases, Duke-NUS Medical School, Singapore
[c] Department of Infectious Diseases, Singapore General Hospital, Singapore
[d] Duke-NUS Medical School, Singapore
[e] Center for Quantitative Medicine, Duke-NUS Medical School, Singapore
[f] Department for International Health, University of Tampere, 33100, Finland
[g] Department of Microbiology and Immunology, Yong Loo Lin School of Medicine, National University of Singapore, Singapore
[h] Antimicrobial Resistance Interdisciplinary Research Group, Singapore-MIT Alliance for Research & Technology (SMART), Singapore

ARTICLE INFO

Keywords:
Measles mumps and rubella (MMR) vaccine
Genomic signature
Sterilizing immunity
Early T-cells response
Cellular immunity

ABSTRACT

Vaccination is an effective approach to reduce disease burden. High vaccination coverage blocks pathogen transmission to ensure herd immunity. However, the concept of herd immunity assumes that vaccinated individuals cannot be infected and mediate silent pathogen transmission. While the correlates of vaccine-mediated protection against disease have been examined, the correlates of sterilizing immunity that prevents infection have not been systematically defined. Here, we used full genome expression profiling to explore the molecular correlates of serological response and non-response to measles, mumps and rubella (MMR) vaccination as surrogates of infection and sterilizing immunity, respectively. We observed that the antibody titers needed to sterilize infection with the vaccine strains were higher than current WHO disease protection thresholds. In subjects with baseline antibodies below such sterilizing immunity thresholds, serological non-response to MMR vaccination was associated with gene expression profile indicative of early T-cell activation and signalling. Specifically, genes that regulate T-cell function and response were induced at day 1 post-vaccination in non-responders but not in responders. These findings suggest that rapid T-cell response prevented MMR vaccine infection to limit antigenic presentation and hence serological response. Collectively, our findings suggest an important role for T-cells in engendering sterilizing immunity.

1. Introduction

Vaccines are effective public health tools in curbing the spread of infectious diseases. They have also led to the successful eradication of smallpox and elimination of poliomyelitis from most parts of the world. Vaccination programs are directed primarily at disease prevention but not necessarily at preventing infection. Indeed, demonstration of vaccine efficacy in late phase clinical trials for dengue, malaria and rotavirus vaccines uses reduced disease incidence relative to placebo as a primary endpoint (Villar et al., 2015; Rts et al., 2012; Madhi et al., 2012). Consequently, to guide development of new vaccines, much focus is placed on defining the correlates of protection from disease, but not infection.

In addition to preventing disease, high vaccination coverage of a population can also result in herd immunity by halting the chain of transmission to susceptible individuals. The level of vaccination coverage that leads to induction of herd immunity is dependent on the basic reproductive rate (R_0) of a pathogen, or the number of secondary cases generated by an infectious individual in a susceptible population. Accordingly, the level of vaccination coverage required to achieve herd immunity, or the herd immunity threshold, increases with R_0. The concept of herd immunity is thus predicated on the assumption that vaccinated individuals are protected against infection and not just disease. In the context of populations with high vaccine coverage, silent pathogen transmission through asymptomatic infections would not pose a problem. However, with reduced vaccine coverage or waning

* Corresponding author. Department of Infectious Diseases, Singapore General Hospital Academia Level 3, 20 College Rd, Singapore 169856, Singapore.
E-mail address: jenny.low@singhealth.com.sg (J.G. Low).

https://doi.org/10.1016/j.antiviral.2019.03.013
Received 12 February 2019; Received in revised form 21 March 2019; Accepted 27 March 2019
Available online 30 March 2019

immunity levels, re-emergence and endemicity of pathogens, especially those with high R_0, could be amplified by this pool of asymptomatically infected vaccinated individuals (Dayan et al., 2008; Shah et al., 2018; Barskey et al., 2012). Indeed, a recent study found that vaccinated individuals can be infected and even develop measles, although the disease is less severe than in unvaccinated individuals (Cherry and Zahn, 2018). Consequently, achieving and sustaining sterilizing immunity that abrogates infection could be important in such instances to prevent re-emergence of vaccine-preventable diseases.

As preventing infection is not a primary goal of most vaccination programs, the correlates of sterilizing immunity, unlike those of protection, have not been systematically examined. Understanding the correlates of sterilizing immunity may now be urgently needed as vaccine uptake and coverage continue to decrease both in developing and developed worlds. Moreover, adults who received childhood vaccines may have waning of vaccination-acquired immunity, which poses an acute problem amongst healthcare workers (HCWs) as infected HCWs may expose patients who may be vulnerable to infections (Hahne et al., 2016; Bonebrake et al., 2010). To explore the correlates of sterilizing immunity, we capitalised on the measles, mumps and rubella (MMR) live attenuated vaccine (LAV) as a framework. The MMR LAV is given as a trivalent formulation in two doses. The MMR vaccine has been in use for more than 30 years and its inclusion in childhood immunization programmes led to ≥99% reduction in these three diseases (Roush et al., 2007; Peltola et al., 2008). Despite the potency of MMR LAV, it is becoming evident in recent decades that adaptive immunity wanes with time. Coupled with reduced vaccine uptake rates fuelled by misinformation and fear-mongering, measles, mumps and rubella (Roush et al., 2007; Peltola et al., 2008) have rapidly re-emerged especially in developed countries across Europe and North America (Davidkin et al., 2008; Kontio et al., 2012; Lewnard and Grad, 2018). We thus took advantage of a MMR catch-up vaccination exercise among HCWs and combined statistical modelling with whole blood genome profiling to identify correlates of sterilizing immunity. Our findings suggest that antibody titers significantly higher than previously recommended are needed to ensure sterilizing immunity; at lower antibody titers, sterilizing immunity remain possible but only in those individuals that showed signatures of early memory T-cell responses.

2. Material and methods

2.1. Study design

Due to the increasing concern of resurgence of measles and mumps cases among HCWs globally (Hahne et al., 2016; Bonebrake et al., 2010), HCWs who were unable to produce laboratory evidence of MMR vaccination history were offered vaccination (GSK Priorix) at no cost by the Singapore General Hospital. Whole blood and sera were collected at baseline (i.e. day 0 before MMR vaccination), day 1 and 3 post-vaccination. Sera was also collected at day 30 post-vaccination to determine antibody response. Study approval was obtained from the Central Institutional Review Board of the Singapore Health Services (CIRB 2017/2374). All participants gave written informed consent.

2.2. Elisa

IgG titers for measles, mumps and rubella at baseline and one month post-vaccination were measured using commercial enzyme immunoassays according to the manufacturer's instructions (Euroimmun).

2.3. Logistic regression model

Using known reproductive numbers (R0) for measles, mumps and rubella (Anderson and May, 1982; Fine, 1993), we used equation P = (1-(1/R0))*100 to define the percentage of the population that needs to be vaccinated to establish herd immunity. We assumed that the susceptible population is defined by subjects with a probability of an IgG boost (> 2-fold) following vaccination. A univariable logistic regression analysis was performed with baseline antibody titers as the independent variable and > 2 fold increase in antibody titer at 1 month post-vaccination as the outcome (Supplementary Table 3). The equation of the logistic curve:

$$log_e\left(\frac{p_i}{1-p_i}\right) = \beta_0 + \beta_1 x_1$$

was then used to derive the baseline antibody titers required for sterilizing immunity in our cohort.

2.4. Mixture model

The mixture model approach estimates the least number of components (groups) that can most parsimoniously describe the baseline antibody titer distribution in a group of subjects. Model fitting of normalized probability density functions was simulated to describe the distribution of baseline antibody titers for measles, mumps and rubella in our study cohort. We used the chi-square statistical test to compare the deviance of each increasingly complex, nested model to that of its immediate simpler predecessor, to enable the simplest fit with the smallest number of component distributions. To minimize over- or under-fitting, model selection tests such as the log likelihood test and Bayesian Information Criterion was performed on models with different number of components to determine the best fit model for distribution of baseline antibody titers for measles, mumps and rubella.

2.5. Genomic expression profiling

RNA isolation from whole blood was performed using the Tempus Spin RNA Isolation kit (Thermo Scientific) according to manufacturer's instructions. RNA samples were subjected to Bioanalyzer assessment before microarray analysis or Nanostring profiling, both of which are further detailed in the Supplementary Methods. Microarray data was deposited in the ArrayExpress database under accession number E-MTAB-7055. Counts were quantile-normalized and $\log_2$ fold change in gene expression on day 1 and day 3 relative to day 0 was calculated for each subject. Analysis of microarray and nCounter data was performed using Partek Genomics Suite 7.18 (Partek Inc.).

2.6. Gene set enrichment analysis (GSEA)

Gene set enrichment analysis (GSEA) is a computational method that assesses whether an *a priori* defined set of genes shows statistically significant differences between two biological phenotypes (Subramanian et al., 2005). We performed GSEA using gene sets from the Reactome and Blood Transcriptomics Module database (Li et al., 2014) to compare enriched pathways in vaccinees. We first ranked genes in the data set based on correlation to the phenotype of interest, which in this study is serological response or non-response to MMR. Next, the rank positions of all members of the gene set were identified and an enrichment score which represents the difference between observed ranking and expected ranking assuming a random rank distribution was calculated for each gene set. Sample labels were then randomized and retested iteratively for enrichment across random classes. Enrichment scores for each gene set across true and random classes were then compared and gene sets that significantly outperformed the random class permutations were considered significant under GSEA. Gene sets were considered significantly enriched if their normalized enrichment score (NES) had a false discovery rate (FDR) q-value of less than 0.05 (Subramanian et al., 2005).

2.7. Viremia assessment with qRT-PCR

RNA from sera was extracted using the Roche MagNA Pure 24 Total NA Isolation Kit, according to manufacturer's instructions, followed by real-time qPCR using previously published Taqman primer and probe sequences for measles, mumps and rubella virus on the Roche LC96 RT-PCR system (Hubschen et al., 2008; Rota et al., 2013). Synthetic RNA standards were prepared by *in vitro* transcription of the N gene for measles and mumps virus, and p150 gene for rubella virus. The limit of detection for measles, mumps and rubella virus was determined to be 100, 10, and 100 genome copies/ul respectively.

2.8. Statistics

Data are represented as mean ± SEM. Statistical analyses were performed using Prism 7.0 software (GraphPad Software Inc.). Significance for paired comparison was determined by paired *t*-test, otherwise unpaired *t*-test was used to test for significance. P values of less than 0.05 were considered statistically significant.

3. Results

3.1. Antibody titers required for sterilizing immunity

A total of 98 HCWs were prospectively enrolled following written informed consent over a period of three months (Supplementary Fig. 1, Supplementary Table 1). Serological status at pre-vaccination (baseline) and at 1 month post-vaccination are shown in Supplementary Table 2. At baseline, the seropositive rates for measles, mumps and rubella were 90.7%, 79.4% and 89.7%, respectively (Supplementary Table 2). The lower seropositive rate for mumps is consistent with what has been reported in other populations (Davidkin et al., 2008; Cardemil et al., 2017). Following vaccination, the seropositive rates of measles, mumps and rubella increased to 94.8% (92 subjects), 91.8% (89 subjects) and 100% (97 subjects), respectively (Supplementary Table 2). Mean antibody titers against each of the three viruses also increased post-vaccination, with the largest fold increase in antibody titers observed for rubella (Supplementary Table 2). In our study, age had no effect on baseline or 1 month post-vaccination IgG titers specific for measles, mumps or rubella (Supplementary Fig. 2).

To explore the relationship between baseline antibody titers and sterilizing immunity, we first tested the serum samples for measles virus (MeV), mumps virus (MuV) and rubella virus (RV) RNA genomes using qRT-PCR. None tested qRT-PCR positive, which is consistent with previous reports that have assessed MMR vaccine viremia (Bosma et al., 1995; Strebel et al., 2008). We thus employed a serological approach to define sterilizing immunity. We considered > 2 fold increase in antibody titer post-vaccination as indicative of non-sterilizing immunity, as such a change has been previously shown to be associated with subclinical infection (Schiff et al., 1985; Whittle et al., 1999). Silent infection and transmission would thus be prevented if the proportion of subjects with a ≤2 fold rise in IgG titers post-vaccination is equal or greater than the herd immunity threshold (levels calculated from the basic reproductive rate, R_0) for the respective viruses (Supplementary Table 3) (Anderson and May, 1982; Fine, 1993; Fine et al., 2011). Through this approach, we found that the estimated baseline measles, mumps and rubella antibody titers required to prevent infection were greater than the manufacturer's cut-offs for seropositivity (Fig. 1). These thresholds were benchmarked through studies that explored correlates of disease protection (Chen et al., 1990; Davis et al., 1971). Among the three viruses, the greatest difference between WHO recommended seropositivity threshold and our estimated baseline titers needed for sterilizing immunity was rubella (Fig. 1).

A binary classification for sterilizing immunity, however, may be too simplistic and misses nuances in immune responses to these viruses. To obtain deeper insight into the determinants of sterilizing immunity, we employed a mixture model approach to determine the number of components required to unbiasedly describe the baseline antibody titer and the associated response post-vaccination for each virus. We found three components as the best fit model using the model selection tests, log likelihood test and Bayesian information criterion, to describe the distribution of baseline antibody titers to measles, mumps and rubella (Fig. 2A, Supplementary Tables 4–6); a binary classification of serostatus did not fit our data. Moreover, the sterilizing immunity threshold we identified in Fig. 1 separated components-2 and -3 for mumps and rubella (Fig. 2A). Increased antibody titers post-vaccination was most clearly observed in component-1 and -2 subjects (Fig. 2B). This corroborated with subjects in components-1 and -2 responding with > 2 fold rise in antibody titer at day 30 post-vaccination (Fig. 2C). In contrast, subjects in component-3 exclusively responded with ≤2 fold rise in antibody titer, with the exception of one subject with > 2 fold rise in antibody titer against mumps (Fig. 2C). Among component-1 and -2 subjects, baseline seropositive subjects for measles, mumps and rubella could be further separated into responders and non-responders to vaccination. We defined responders as those with > 2-fold rise in antibody titers for either measles, mumps or rubella, and non-responders as those with ≤2-fold increase in antibody titers for measles, mumps and rubella at day 30 post-vaccination. Consistent with the R_0 analysis (Fig. 1A), the proportion of seropositive subjects with a > 2-fold rise in antibody titers was also highest for rubella (23%) (Fig. 2C, Supplementary Table 2), as compared to measles (2.3%) and mumps (9.1%).

3.2. Distinct host response differentiates serological responders from non-responders

Seropositive subjects with baseline antibody titers below component 3 are interesting; these subjects have similar baseline antibody titers yet only some but not all responded serologically to vaccination. To understand the molecular basis for this lack of serological change post-vaccination, we compared non-responders with responders. Host responses were explored using whole genome microarray analysis with RNA extracted from whole blood. As the number of differentially expressed genes following baseline normalization was small (Supplementary Fig. 3), we used gene set enrichment analysis (GSEA) to identify enriched molecular pathways (Subramanian et al., 2005). We first performed GSEA using the Reactome database, which provides curated pathways of DNA replication, translation, metabolism, signal transduction and other cellular processes. While differences in enrichment were observed at day 1 and day 3 post-vaccination (Fig. 3, Supplementary Fig. 4), a greater number of significantly enriched transcriptomic modules were identified on day 1 post-vaccination (Fig. 3, Supplementary Table 7). We thus focused our attention on the transcriptomic differences between day 1 post-vaccination and baseline. The top most enriched gene set in non-responders relative to responders was adaptive immune system (Fig. 3A). In contrast, none of the Reactome pathways were positively enriched in responders.

To gain a better resolution on the types of gene expression changes in the adaptive immune system, we repeated GSEA using blood transcription modules (BTMs). BTMs were developed to interrogate the transcriptional signatures of human vaccines and are representative of many known gene signatures for immune cell types and vaccine-mediated immunological processes (Li et al., 2014). Strikingly, of the 10 positively enriched gene sets, 5 were associated with T cell markers, T cell activation and signalling (Fig. 3B). Other enriched gene sets included NK cell markers, antigen presentation and B cell markers (Fig. 3B). Likewise, only one BTM for neutrophil markers was positively enriched in responders (Fig. 3B).

As GSEA enrichment scores reflect the degree to which a gene set is overrepresented in non-responders against responders, we next compared gene expression of leading edge genes driving enrichment of the Reactome adaptive immune system pathway and the top 3 T-cell

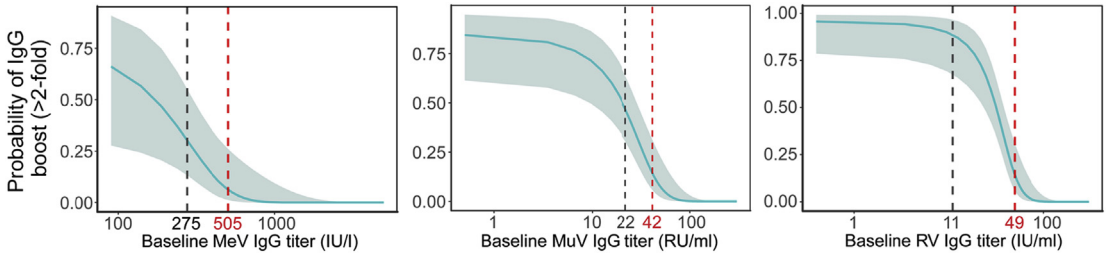
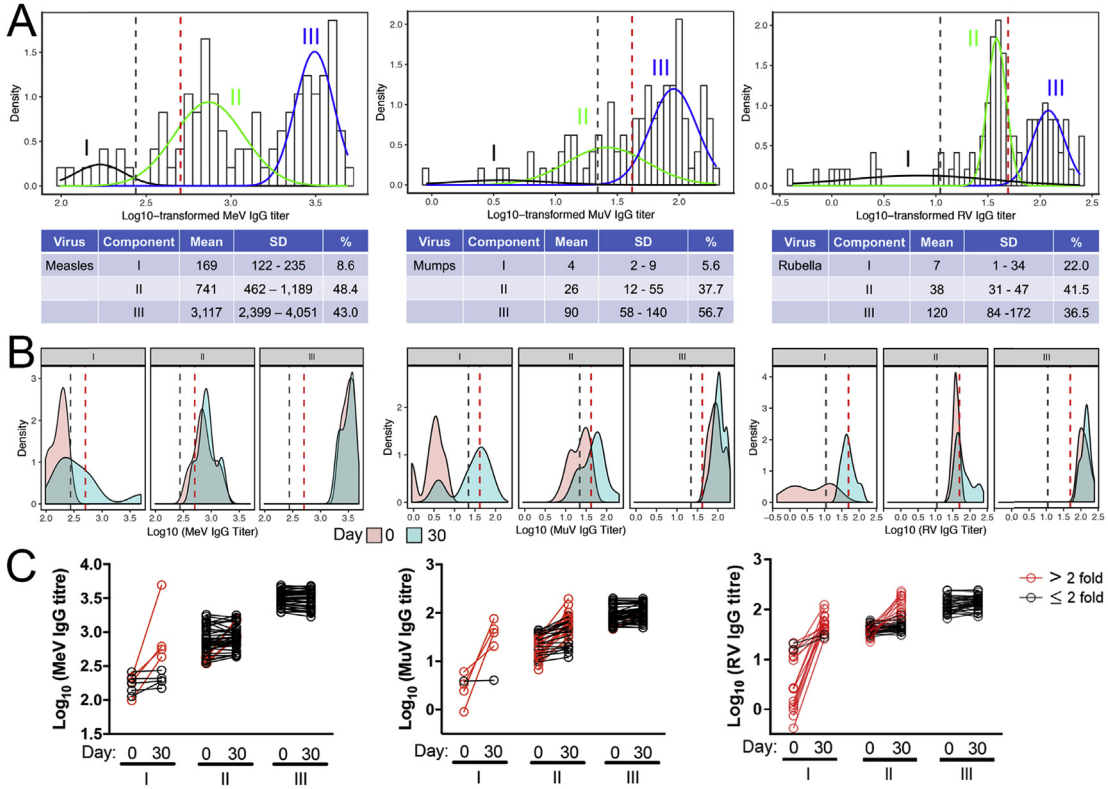


Fig. 1. Estimated antibody thresholds for sterilizing immunity are higher than current WHO thresholds for disease protection. Logistic regression curve models the relationship between baseline antibody titer and a > 2-fold increase in post-vaccination antibody titer in our study participants. Black dotted lines: manufacturer's positive cut-offs for seropositivity, red dotted lines: estimated antibody thresholds for sterilizing immunity.



Virus	Component	Mean	SD	%
Measles	I	169	122 - 235	8.6
	II	741	462 – 1,189	48.4
	III	3,117	2,399 – 4,051	43.0

Virus	Component	Mean	SD	%
Mumps	I	4	2 - 9	5.6
	II	26	12 - 55	37.7
	III	90	58 - 140	56.7

Virus	Component	Mean	SD	%
Rubella	I	7	1 - 34	22.0
	II	38	31 - 47	41.5
	III	120	84 -172	36.5

Fig. 2. Increased antibody titers following MMR vaccination. (A) Density histograms of baseline antibody titers for measles virus (MeV), mumps virus (MuV) and rubella virus (RV), showing fit results for 3-component mixture models. The black, green and blue solid lines represent normalized probability density functions for components −1, −2 and −3. Summary statistics indicate mean (± SD) antibody titer for each component and percentage of subjects that belong to each component. MeV, MuV, RV model: $p < 0.0001$, chi-square statistical test comparing three components in the fitted model. (B) Shift in antibody titers against measles, mumps and rubella following vaccination. Black and red dotted lines indicate manufacturer's positive cut-offs for seropositivity and antibody threshold for sterilizing immunity respectively. (C) Change in antibody titers for individual subjects following MMR vaccination. Greater than two-fold increase in antibody titers at day 30 post-vaccination are indicated in red.

associated BTMs. Analysis of leading edge genes within these positively enriched pathways show distinct differences in expression between non-responders and responders (Fig. 3C and D). The divergent gene expression in responders and non-responders was validated using an alternative method, the nCounter RNA profiling platform which provides digital counts of mRNA transcripts for a panel of 579 immune genes. We observed a statistically significant correlation between the expression of this panel of genes as measured by nCounter compared to microarray (Supplementary Fig. 5). Likewise, genes in the Reactome adaptive immune system pathway and T-cell associated BTMs were similarly upregulated in non-responders relative to responders (Fig. 3E and F).

To further explore the notion that T-cell response was an important feature in non-responders, we examined the differences in expression of T-cell associated genes at day 1 post-vaccination compared to baseline. Interestingly, several genes that are known to play an important role in regulating T-cell function showed significantly increased expression in non-responders but not in responders. Specifically, MHC Class II HLA-DRB3 and HLA-DRA (Fig. 4A and B), and the T-cell co-stimulatory molecule CD86 (Fig. 4C) were induced in non-responders but not responders. Likewise, several other genes known to play roles in antigen presentation and T-cell response were also similarly induced in non-responders but not in responders: CYBB (Fig. 4E), also known as NADPH oxidase 2 (NOX2), which is known for its role in antigen processing in the phagosome; the chemokine receptor CCR2 which is a positive regulator of T-cell activation and migration (Fig. 4F); as well as CD74 which is thought to be involved in memory T-cell survival and homeostasis (Fig. 4D) (Weng et al., 2012). These findings suggest that early cell-mediated immune response enables sterilizing immunity when antibody titers are not sufficiently high to prevent infection.

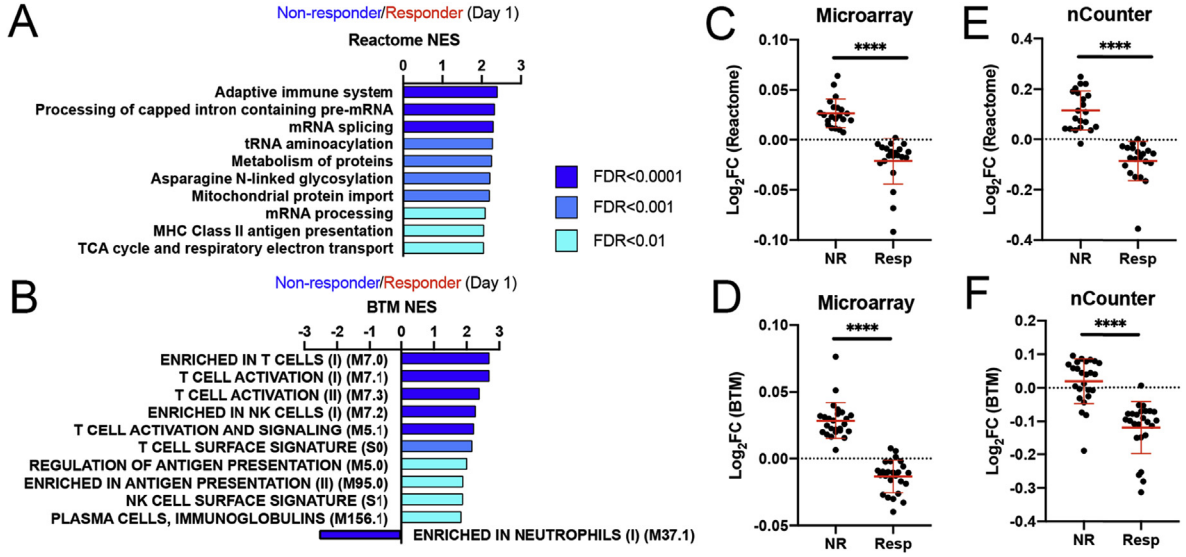


Fig. 3. Transcriptomic induction of T-cell associated gene sets post-vaccination differentiates non-responders from responders. (A–B) Normalized enrichment scores (NES) for positively and negatively enriched gene sets between non-responders (n = 7) and responders (n = 7) at day 1 post-vaccination. GSEA was performed using the Reactome database (A) or blood transcriptomic modules (B). (C, E) Average expression fold-change of leading edge genes driving enrichment of adaptive immune system pathway of the Reactome database following microarray analysis (C) and nCounter digital RNA profiling (E) on day 1 post-vaccination. (D, F) Average expression fold-change of leading edge genes driving enrichment of top 3 T-cell related BTMs following microarray analysis (D) and nCounter digital RNA profiling (F) on day 1 post-vaccination. Each point represents one gene. ****p < 0.0001, unpaired *t*-test. Error bars indicate standard deviation.

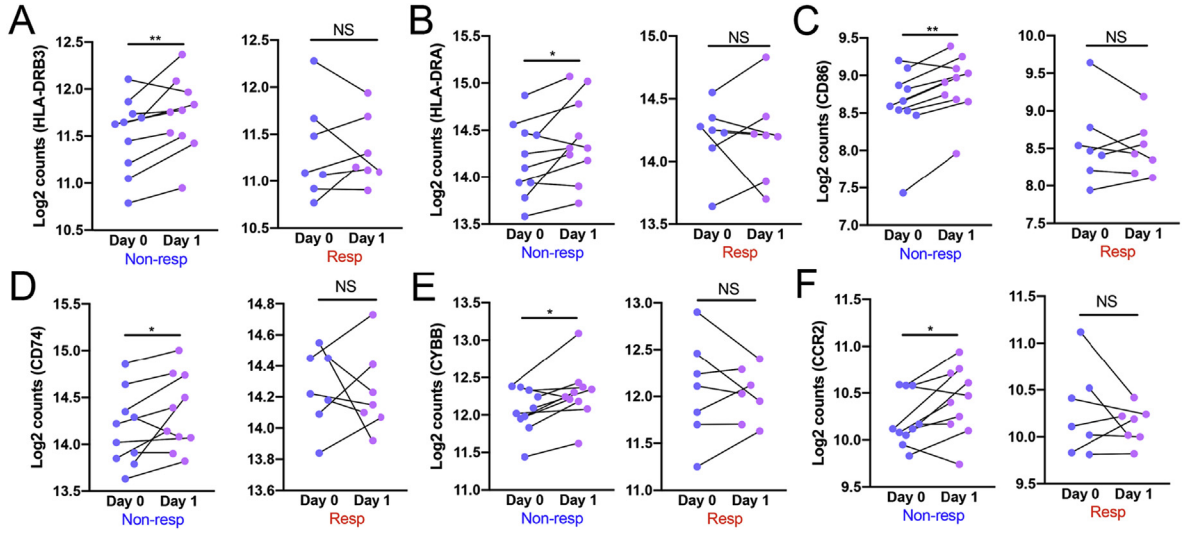


Fig. 4. Genes involved in T cell function and response are upregulated in non-responders at 1 day post-vaccination. Pairwise comparison of gene expression following nCounter profiling at day 0 (baseline) and day 1 post-vaccination in non-responders (n = 10) and responders (n = 7). Each pair represents one subject. **p < 0.01, *p < 0.05, paired *t*-test. NS, not significant.

4. Discussion

The correlates of sterilizing immunity is a neglected area of vaccinology. Large scale vaccine trials necessarily rely on case observation to determine efficacy endpoints, as evidence of reduced pathogen transmission requires costly laboratory support. Our goal in this study is thus to combine statistical modelling with whole genome profiling to derive descriptive host-response data that forms a basis for future hypothesis-driven studies.

The immune response to revaccination has been studied mostly in the context of prime-boost regimens for subunit protein vaccines. Theoretically, memory B- and T-cells are induced during priming and are then re-stimulated upon boosting to result in higher magnitude of adaptive immune response. LAV boosters are thought to work in a similar manner to sustain vaccine efficacy by stimulating anamnestic humoral and cellular responses (Hahne et al., 2016; Crotty et al., 2003; Kongsgaard et al., 2017; Date et al., 2008). Serology has been the more widely used approach to assess adaptive immune response to vaccination and revaccination as well as population level immunity. Indeed, a majority of the currently licensed vaccines elicit antibody response against the immunogen; none of these vaccines are directed at eliciting T-cell response only. Consequently, serological assays have been useful as tools for vaccine development and population immunity monitoring. In contrast, cellular immunity assays have been applied mostly in smaller scale immunological studies rather than as tools to assess population level immunity. Our findings of early T-cell activation in serological non-responders to vaccination suggests a need for population level study into T-cell mediated immunity.

Using genomic tools, we found gene expression changes suggestive of T-cell activation and antigen presentation in subjects that were

seropositive at baseline but who then showed a ≤ two-fold rise in antibody titer post-vaccination; a ≤ two-fold rise in antibody titer post-vaccination/infection has been used as an indicator for sterilizing immunity (Schiff et al., 1985; Whittle et al., 1999). In addition to the adaptive immune system Reactome pathway, more than 50 other Reactome pathways were enriched at day 1 post-vaccination in non-responders (Supplementary Table 7), including pathways responsible for cellular metabolism, gene transcription and protein synthesis, supporting the view that transcriptional and metabolic reprogramming occurs rapidly upon T-cell activation, to enable T-cell proliferation and synthesis of effector molecules (Shehata et al., 2017; Bantug et al., 2018). We postulate that rapid adaptive immune response prevented or abrogated LAV replication to limit antigenic presentation and hence restrain differentiation of memory B-cells to antibody-producing plasma cells (Kongsgaard et al., 2017). Positive MMR viremia finding would have been useful to support this notion although our inability to detect viremia is consistent with findings from previous studies that found none of the three components in the MMR vaccine elicited detectable viremia (Bosma et al., 1995; Strebel et al., 2008). However, our interpretation of the data is in agreement with what has previously been observed following immunization with the live attenuated influenza vaccine, whereby anamnestic T-cell responses were stimulated without concomitant increase in antibody titers during LAV boosting (Mohn et al., 2017).

Besides suggesting a more widespread application of T-cell assays for vaccine studies, our findings also hint at additional possibilities. Firstly, significant change in serological titers upon revaccination with a LAV should perhaps be interpreted as inadequate protection against infection. This could be used to down-select vaccine candidates without proceeding to large scale phase 3 efficacy trials. This is especially since re-vaccination in the clinical development stages would likely take place soon after completion of primary vaccination, well before antibody titers wane to potentially sub-protective levels. If indeed a vaccine is unable to prevent infection within such a short time, it would not be an ideal candidate as a long-term preventative measure for public health application. Secondly, periodic sampling of vaccinated populations for waning immunity could be a strategy to monitor herd immunity at the population level. The proportion of subjects that react to revaccination with recall of memory adaptive immunity should reflect the threshold for herd immunity needed to prevent disease re-emergence. When this proportion is less than expected for herd immunity, revaccination programs could then be implemented before disease re-emergence. Such a strategy could also be useful to establish the duration of protection from existing and new LAV vaccines.

A limitation of our study is the lack of lymphocyte flow cytometric analysis as we had set out to explore the correlates of sterilizing immunity in order to provide a genomic basis for more hypothesis-driven studies. Future studies that characterize the T-cell response in protection against infection will be needed to shed further insights into the underpinnings of sterilizing immunity.

In conclusion, our findings suggest a key role for adaptive cellular immune response in engendering sterilizing immunity.

Conflict of interest

The authors have declared that no conflict of interest exists.

Acknowledgements

JGL and EEO are funded by the National Medical Research Council of Singapore, through the Clinician-Scientist Awards. This study was funded by a generous gift from the Tanoto Foundation and the Singapore Infectious Diseases Initiative Bridging Grant from the Ministry of Health -Singapore. We also thank the nurses and study volunteers at the Singapore General Hospital.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.antiviral.2019.03.013.

References

Anderson, R.M., May, R.M., 1982. Directly transmitted infections diseases: control by vaccination. Science 215 (4536), 1053–1060.
Bantug, G.R., Galluzzi, L., Kroemer, G., Hess, C., 2018. The spectrum of T cell metabolism in health and disease. Nat. Rev. Immunol. 18 (1), 19–34.
Barskey, A.E., Schulte, C., Rosen, J.B., Handschur, E.F., Rausch-Phung, E., Doll, M.K., et al., 2012. Mumps outbreak in orthodox jewish communities in the United States. N. Engl. J. Med. 367 (18), 1704–1713.
Bonebrake, A.L., Silkaitis, C., Monga, G., Galat, A., Anderson, J., Trad, J.T., et al., 2010. Effects of mumps outbreak in hospital, Chicago, Illinois, USA, 2006. Emerg. Infect. Dis. 16 (3), 426–432.
Bosma, T.J., Corbett, K.M., O'Shea, S., Banatvala, J.E., Best, J.M., 1995. PCR for detection of rubella virus RNA in clinical samples. J. Clin. Microbiol. 33 (5), 1075–1079.
Cardemil, C.V., Dahl, R.M., James, L., Wannemuehler, K., Gary, H.E., Shah, M., et al., 2017. Effectiveness of a third dose of MMR vaccine for mumps outbreak control. N. Engl. J. Med. 377 (10), 947–956.
Chen, R.T., Markowitz, L.E., Albrecht, P., Stewart, J.A., Mofenson, L.M., Preblud, S.R., et al., 1990. Measles antibody: reevaluation of protective titers. J. Infect. Dis. 162 (5), 1036–1042.
Cherry, J.D., Zahn, M., 2018. Clinical characteristics of measles in previously vaccinated and unvaccinated patients in California. Clin. Infect. Dis. 67 (9), 1315–1319.
Crotty, S., Felgner, P., Davies, H., Glidewell, J., Villarreal, L., Ahmed, R., 2003. Cutting edge: long-term B cell memory in humans after smallpox vaccination. J. Immunol. 171 (10), 4969–4973.
Date, A.A., Kyaw, M.H., Rue, A.M., Klahn, J., Obrecht, L., Krohn, T., et al., 2008. Long-term persistence of mumps antibody after receipt of 2 measles-mumps-rubella (MMR) vaccinations and antibody response after a third MMR vaccination among a university population. J. Infect. Dis. 197 (12), 1662–1668.
Davidkin, I., Jokinen, S., Broman, M., Leinikki, P., Peltola, H., 2008. Persistence of measles, mumps, and rubella antibodies in an MMR-vaccinated cohort: a 20-year follow-up. J. Infect. Dis. 197 (7), 950–956.
Davis, W.J., Larson, H.E., Simsarian, J.P., Parkman, P.D., Meyer Jr., H.M., 1971. A study of rubella immunity and resistance to infection. JAMA 215 (4), 600–608.
Dayan, G.H., Quinlisk, M.P., Parker, A.A., Barskey, A.E., Harris, M.L., Schwartz, J.M., et al., 2008. Recent resurgence of mumps in the United States. N. Engl. J. Med. 358 (15), 1580–1589.
Fine, P.E., 1993. Herd immunity: history, theory, practice. Epidemiol. Rev. 15 (2), 265–302.
Fine, P., Eames, K., Heymann, D.L., 2011. "Herd immunity": a rough guide. Clin. Infect. Dis. 52 (7), 911–916.
Hahne, S.J., Nic Lochlainn, L.M., van Burgel, N.D., Kerkhof, J., Sane, J., Yap, K.B., et al., 2016. Measles outbreak among previously immunized healthcare workers, The Netherlands, 2014. J. Infect. Dis. 214 (12), 1980–1986.
Hubschen, J.M., Kremer, J.R., De Landtsheer, S., Muller, C.P., 2008. A multiplex TaqMan PCR assay for the detection of measles and rubella virus. J. Virol. Methods 149 (2), 246–250.
Kongsgaard, M., Bassi, M.R., Rasmussen, M., Skjodt, K., Thybo, S., Gabriel, M., et al., 2017. Adaptive immune responses to booster vaccination against yellow fever virus are much reduced compared to those after primary vaccination. Sci. Rep. 7 (1), 662.
Kontio, M., Jokinen, S., Paunio, M., Peltola, H., Davidkin, I., 2012. Waning antibody levels and avidity: implications for MMR vaccine-induced protection. J. Infect. Dis. 206 (10), 1542–1548.
Lewnard, J.A., Grad, Y.H., 2018. Vaccine waning and mumps re-emergence in the United States. Sci. Transl. Med. 10 (433).
Li, S., Rouphael, N., Duraisingham, S., Romero-Steiner, S., Presnell, S., Davis, C., et al., 2014. Molecular signatures of antibody responses derived from a systems biology study of five human vaccines. Nat. Immunol. 15 (2), 195–204.
Madhi, S.A., Kirsten, M., Louw, C., Bos, P., Aspinall, S., Bouckenooghe, A., et al., 2012. Efficacy and immunogenicity of two or three dose rotavirus-vaccine regimen in South African children over two consecutive rotavirus-seasons: a randomized, double-blind, placebo-controlled trial. Vaccine 30 (Suppl. 1), A44–A51.
Mohn, K.G.I., Zhou, F., Brokstad, K.A., Sridhar, S., Cox, R.J., 2017. Boosting of cross-reactive and protection-associated T cells in children after live attenuated influenza vaccination. J. Infect. Dis. 215 (10), 1527–1535.
Peltola, H., Jokinen, S., Paunio, M., Hovi, T., Davidkin, I., 2008. Measles, mumps, and rubella in Finland: 25 years of a nationwide elimination programme. Lancet Infect. Dis. 8 (12), 796–803.
Rota, J.S., Rosen, J.B., Doll, M.K., McNall, R.J., McGrew, M., Williams, N., et al., 2013. Comparison of the sensitivity of laboratory diagnostic methods from a well-characterized outbreak of mumps in New York city in 2009. Clin. Vaccine Immunol. 20 (3), 391–396.
Roush, S.W., Murphy, T.V., Vaccine-Preventable Disease Table Working G, 2007. Historical comparisons of morbidity and mortality for vaccine-preventable diseases in the United States. JAMA 298 (18), 2155–2163.
Rts, S.C.T.P., Agnandji, S.T., Lell, B., Fernandes, J.F., Abossolo, B.P., Methogo, B.G., et al., 2012. A phase 3 trial of RTS,S/AS01 malaria vaccine in African infants. N. Engl. J. Med. 367 (24), 2284–2295.
Schiff, G.M., Young, B.C., Stefanovic, G.M., Stamler, E.F., Knowlton, D.R., Grundy, B.J.,

et al., 1985. Challenge with rubella virus after loss of detectable vaccine-induced antibody. Rev. Infect. Dis. 7 (Suppl. 1), S157–S163.
Shah, M., Quinlisk, P., Weigel, A., Riley, J., James, L., Patterson, J., et al., 2018. Mumps outbreak in a highly vaccinated university-affiliated setting before and after a measles-mumps-rubella vaccination campaign-Iowa, July 2015-May 2016. Clin. Infect. Dis. 66 (1), 81–88.
Shehata, H.M., Murphy, A.J., Lee, M.K.S., Gardiner, C.M., Crowe, S.M., Sanjabi, S., et al., 2017. Sugar or fat?-metabolic requirements for immunity to viral infections. Front. Immunol. 8, 1311.
Strebel, P.M.P.M., Dayan, G.H., Haalsey, N.A., 2008. In: Plotkin, S.O.W., Offit, P. (Eds.), Vaccines. Elsevier, Philadelphia, PA, pp. 353–398.
Subramanian, A., Tamayo, P., Mootha, V.K., Mukherjee, S., Ebert, B.L., Gillette, M.A., et al., 2005. Gene set enrichment analysis: a knowledge-based approach for interpreting genome-wide expression profiles. Proc. Natl. Acad. Sci. U. S. A. 102 (43), 15545–15550.
Villar, L., Dayan, G.H., Arredondo-Garcia, J.L., Rivera, D.M., Cunha, R., Deseda, C., et al., 2015. Efficacy of a tetravalent dengue vaccine in children in Latin America. N. Engl. J. Med. 372 (2), 113–123.
Weng, N.P., Araki, Y., Subedi, K., 2012. The molecular basis of the memory T cell response: differential gene expression and its epigenetic regulation. Nat. Rev. Immunol. 12 (4), 306–315.
Whittle, H.C., Aaby, P., Samb, B., Jensen, H., Bennett, J., Simondon, F., 1999. Effect of subclinical infection on maintaining immunity against measles in vaccinated children in West Africa. Lancet 353 (9147), 98–102.