Current perspectives in assessing humoral immunity after measles vaccination

Iana H. Haralambieva, Richard B. Kennedy, Inna G. Ovsyannikova, Daniel J. Schaid & Gregory A. Poland

REVIEW

Check for updates

Current perspectives in assessing humoral immunity after measles vaccination

Iana H. Haralambieva[a], Richard B. Kennedy[a], Inna G. Ovsyannikova[a], Daniel J. Schaid[a,b] and Gregory A. Poland[a]

[a]Mayo Clinic Vaccine Research Group, Mayo Clinic, Rochester, MN, USA; [b]Department of Health Sciences Research, Mayo Clinic, Rochester, MN, USA

ABSTRACT

Introduction: Repeated measles outbreaks in countries with relatively high vaccine coverage are mainly due to failure to vaccinate and importation; however, cases in immunized individuals exist raising questions about suboptimal measles vaccine-induced humoral immunity and/or waning immunity in a low measles-exposure environment.

Areas covered: The plaque reduction neutralization measurement of functional measles-specific antibodies correlates with protection is the gold standard in measles serology, but it does not assess cellular-immune or other parameters that may be associated with durable and/or protective immunity after vaccination. Additional correlates of protection and long-term immunity and new determinants/signatures of vaccine responsiveness such as specific *CD46* and *IFI44L* genetic variants associated with neutralizing antibody titers after measles vaccination are under investigation. Current and future systems biology studies, coupled with new technology/assays and analytical approaches, will lead to an increasingly sophisticated understanding of measles vaccine-induced humoral immunity and will identify 'signatures' of protective and durable immune responses.

Expert opinion: This will translate into the development of highly predictive assays of measles vaccine efficacy, effectiveness, and durability for prospective identification of potential low/non-responders and susceptible individuals who require additional vaccine doses. Such new advances may drive insights into the development of new/improved vaccine formulations and delivery systems.

1. Measles in high vaccine coverage settings and measles vaccine failure

Measles is a highly contagious disease, which has been eliminated in the United States for more than **18** years. It is still a common threat in the underdeveloped world with potentially life-threatening sequelae and over 450 known pediatric deaths each day [1]. It was estimated that over 7 million people were infected with measles worldwide in 2016 with 89,780 reported measles-related deaths [2]. Measles outbreaks have been limited in the United States during the last few years and are mainly a result of importation and predominantly involve unvaccinated individuals who travel, as well as their contacts (e.g., 86 reported measles cases in 2016; 118 measles cases in 2017; and 124 measles cases from 22 states in 2018, as of 11 August 2018) [3,4]. In 2017, the World Health Organization announced a four-fold rise in the number of measles cases in Europe (23,927 cases), with large outbreaks affecting more than 15 European countries [5,6]. Furthermore, over 41,000 measles cases were reported in Europe for the first half of 2018 (the most affected countries were France, Georgia, Greece, Italy, the Russian Federation, Serbia, and Ukraine), with at least 37 reported pediatric deaths [5,6]. This resurgence of measles is primarily due to failure to vaccinate and sustain high immunization coverage with the two-dose measles vaccination schedule in the affected countries/regions. However, this is an indication that measles is and will continue to be

a public health concern for both developing and developed countries for the foreseeable future.

Long-term sequelae of measles are more serious and common than previously thought. A recent study assessing national-level information from England, Wales, the United States, and Denmark, from both the pre- and the post-vaccine era, provided statistical/modeling evidence for the association of measles with the long-term increase (approximately 2- to 3-year observed impact) of non-measles infectious disease mortality in children [7]. These non-specific effects of measles on immunity to other pathogens are likely due to measles-induced immunosuppression from lymphocyte depletion of memory B and T lymphocytes and/or from measles-related functional immune impairment [8–11]. Recent research also re-estimates the rate of developing subacute sclerosing panencephalitis/SSPE, a fatal progressive inflammation of the brain resulting from persistent measles virus (MV) infection, to be 1 in 609 after measles disease occurring in infancy [12].

To prevent persisting measles endemicity and target measles for global eradication, achieving and sustaining herd immunity of at least 90–95% is required [13–17]. It is generally accepted that the live attenuated measles vaccine has a high protective efficacy, particularly after two vaccine doses (although it may be lower and not life-long compared to the wild-type virus infection), and a field effectiveness of 94.1% (IQR, 88.3%-98.3%) after two doses [18]. Accordingly, measles

mainly affects unvaccinated individuals. It is also indisputable that a two-dose measles vaccination program must be implemented and sustained globally to reduce measles morbidity/mortality and achieve measles eradication. What is still a subject of debate is the ability of the current measles vaccine to sustain long-term protective immunity and adequate herd immunity in settings with no wild-type virus exposure (i.e., no boosting of immunity resulting from asymptomatic infection).

Primary measles vaccine failure (a failure to develop protective immunity after vaccination) is not uncommon (approximately 2–12% for children immunized at/around one year of age [19–21]) and can be partly managed by the administration of a second dose of vaccine and by increasing the age at first vaccination (in regions with low measles incidence) to ensure immune system maturity and loss of maternally acquired antibodies [22]. The assessment of secondary vaccine failure (waning immunity or failure to sustain protective immunity over time) can be difficult and requires long-term monitoring of measles vaccine-induced adaptive immunity after the first and second vaccine doses and ideally vaccine efficacy data, immunogenicity data, and epidemiological information on measles cases/outbreaks in the geographical area.

Annual measles outbreaks in high vaccine coverage settings also continue to occur. Although these outbreaks typically involve importation and mostly affect unvaccinated individuals, they also reveal surprisingly high numbers of vaccine failure among one- and two-dose recipients of measles-containing vaccine who were infected [15–17,22–53]. In a recently published study of registered laboratory-confirmed measles cases in California between 2000 and 2015, Cherry and Zahn [51] report 232 measles cases with a documented vaccine history, of which 9% (20 cases) were after one dose and 11% (26 cases) were after two doses of measles-containing vaccine (median 16.7 years after last vaccination, range 6 to 23.6 years after last vaccination), indicating the likely occurrence of waning immunity [51]. Earlier studies suggest a secondary measles vaccine failure rate of ~5%, approximately 10–15 years after the second immunization [31,54]. Previous studies have also reported a combination of laboratory methods (measles plaque reduction neutralization assay [PRN], measles IgG avidity assays, and detection of MV RNA by RT-qPCR) and criteria for the classification of measles reinfection cases and secondary vaccine failure [55,56]. Larger and more sophisticated studies are still needed to more precisely estimate secondary vaccine failure rates (waning immunity) in low measles incidence (high vaccine coverage) settings.

2. Immune measures and correlates of protection after measles vaccination

Measles virus cell entry and infection are mediated by the known MV-specific cellular receptors SLAM/CD150, nectin-4/NECTIN4/PVRL4 and CD46 (operational only for attenuated MV strains) [57–60]. Wild-type and attenuated measles viruses elicit differential innate/inflammatory immune responses (NFκB signaling/activation of the NLRP3 inflammasome with no detectable interferon type 1 response for the wild-type MV strains and detectable induction of interferon-stimulated genes [ISGs] for the attenuated MV strains, respectively), but the implications of these differences for the development of adaptive immunity are unclear [61–63]. Protective immunity to measles is accomplished by high-avidity neutralizing antibodies directed to the MV surface glycoproteins, primarily the hemagglutinin/H protein (with a modest contribution of antibodies to the fusion/F protein), which effectively neutralize SLAM-using wild-type MV infection of lymphocytes [54,61,64–67]. Measles vaccine elicits both neutralizing and non-neutralizing antibodies against different MV proteins, as well as measles-specific cellular immunity, with limited correlation between measures of the humoral and cellular arms of immunity [68]. CD4+ T cells are not essential, but they (in particular, the follicular T-helper/Tfh cells in the lymph nodes) can facilitate protective humoral immunity by providing help for the formation of germinal centers, for the activation and differentiation of B cells, and for isotype switching and affinity maturation in antibody-secreting cells/ASCs [69]. CD8+ T cells are considered important for viral clearance [61]. Functional measles-specific neutralizing antibodies after vaccination (anti-H and anti-F) are quantified using a classical plaque reduction neutralization test (PRN) or its improved automated version, the plaque reduction microneutralization (PRMN) assay [70–72], in specialized laboratory settings by trained personnel. Routinely, these assays rely on CD46-mediated *in vitro* infection of Vero cells (that do not express human SLAM) with attenuated Edmonston-based MV strains; and for these reasons, the results may not fully reflect the protective antibody efficacy upon wild virus measles exposure [54,73].

With the above taken into consideration, the currently accepted correlate of protection against measles is a PRN titer of MV-specific neutralizing antibodies >120 (or >120 mIU/mL), which predicts protection from clinical disease [64,71,72,74]. It has been repeatedly shown that serum antibodies (e.g., passively transferred immunoglobulins or transplacentally acquired antibodies) are sufficient to confer protection from measles [54,61,64–67]. However, it has been demonstrated that subjects with low/undetectable PRN antibody levels may still be protected from clinical measles, suggesting a role for cellular immunity in protection [54,61,64–67].

In addition to the PRN assay, seroprevalence studies assessing measles vaccine-induced humoral immunity apply an array of other assays (reviewed in [75–81], including a variety of automated commercial immunoassays (e.g., multiplex microsphere/bead fluorescence-based immunoassays) and microtiter-plate enzyme-linked immunoassays (EIA) reporting qualitative and/or quantitative results. Among the most commonly used are the Enzygnost® Anti-Measles Virus/IgG EIA (Siemens Health Care Diagnostics GmbH, Marburg, Germany) and the Serion Measles IgG EIA (Institut Virion\Serion GmbH, Würzburg, Germany) [75]. With few exceptions (the Enzygnost® Anti-Measles Virus/IgG), these assays were not calibrated against the 2nd WHO international measles standard (the 3rd WHO international measles standard is not currently recommended for EIA calibration [82]). More importantly, EIA assays measure antibodies to other abundant MV proteins (e.g., the N protein in addition to H and F) and have limited ability to measure antibodies to conformational epitopes; thus, their utility for categorization of individuals into immune or non-immune and assessing potential measles susceptibility is limited, particularly at the lower range of antibody titer [75].

A recently developed measles-specific assay using proteome microarray (antibody array) technology successfully detected antibodies against five MV proteins (H, F, N, P, and L) in recipients of measles vaccine, and the measures/patterns were well correlated with the neutralizing antibody response [83].

Antibody avidity assays are also emerging as useful tests for distinguishing primary from secondary humoral immune response during measles outbreaks in high vaccine coverage settings. Avidity is defined as the cumulative strength of attachment/binding of multivalent antibodies to multivalent antigens. A new MV-specific IgG avidity assay was developed by the CDC using a modified commercial EIA assay with the use of the denaturant diethylamine (DEA) and validated with a panel of reference serum samples [84]. This assay provides useful thresholds for classification of antibodies into high/low or intermediate avidity and can supplement IgM antibody assays in the serological assessment of measles cases, as well as facilitate the classification of secondary vaccine failures [84].

Measurement of other markers of measles-specific humoral immunity are also currently being introduced and used, but their outputs (immune outcomes) are not generally accepted as correlates or surrogates of protection against measles. The memory B cell ELISPOT assay uses peripheral blood mononuclear cells (PBMCs) or purified B cells to provide a quantitative measure of the frequencies of pre-existing measles-specific memory B cells after non-specific polyclonal B cell stimulation followed by the enumeration of antigen-specific ASCs [85–89]. Similarly, a plasmablast ELISPOT assay (without non-specific B cell stimulation) directly measures the frequencies of the antigen-specific circulating plasmablasts that peak around day 7 after vaccination [90]. There has been some controversy in the literature over the correlation of measles-specific antibody titers with the frequencies of measles-specific memory B cells and the use of the latter as a predictor of the duration of antibody response/antibody waning [87–89]. Memory B cells are important for a prompt humoral response upon antigen re-exposure, but most likely long-term measles-specific antibody production is maintained by antigen-specific long-lived plasma cells in the bone marrow rather than reactivation/differentiation of memory B cells [91–95]. No feasible assay for large-scale studies exists today to reliably measure the quantity and characteristics of antigen-specific long-lived plasma cells (as well as the antigen-specific Tfh cells) in humans due to their specific niche/homing (ideally assessment requires bone marrow and lymph node biopsies).

Assessment of measles vaccine-induced humoral immunity in vulnerable populations (particularly in pediatric patients suffering from chronic infections and/or immunosuppression) is also important for the maintenance of measles control and eradication/elimination efforts for the general population. A study assessing B cell compartment immunity in 70 HIV-1-infected children established the importance of early antiretroviral therapy for the maintenance of long-term immunity (measles-specific memory B cell frequencies and protective measles antibody titers) after routine vaccinations [96]. Similarly, other conditions associated with immunosuppression (e.g., transplantation and primary immunodeficiencies) often lead to impaired development and/or waning of measles vaccine-induced immunity [97–99]. Monitoring of measles immunity in such cases is critical for the optimization of population vaccination strategies and maintenance of long-term protection against measles.

Addressing the current knowledge and public health gap, as well as barriers to measles elimination activities, requires new approaches in assessing and predicting humoral immune response after measles vaccination in order to prospectively identify vaccine responders and non- or low-responders and/or potentially measles susceptible individuals whose protective titers wane over time. Such approaches and perspectives are in line with the WHO 2012–2020 Global Measles and Rubella Strategic Elimination Plan and the Midterm Review which recommended "research on susceptibility profiles for measles, and research related to outbreaks in high vaccine coverage settings [100], and are reviewed in the sections below with a focus on identifying determinants/signatures of measles vaccine-induced humoral immunity.

3. Contribution of HLA and candidate immune response genes to measles vaccine-induced variations in humoral immunity

Over the last 23 years, our group has investigated the wide inter-individual variation in circulating humoral antibody responses after routine measles vaccination in highly immunized healthy populations and has systematically defined genetic contributions to inter-individual immune response variations and vaccine failure (reviewed in [17,49]).

Both host genetics and environmental factors contribute to variability in immune responses to vaccines. Among the host genetic determinants that are involved in protective immunity against measles are the highly polymorphic HLA and non-HLA genetic variants. In this regard, HLA allelic associations with humoral immune responses after measles vaccination have been studied in detail. Measles vaccine-induced immunity can involve strong HLA class I- and class II-restricted CD8+ and CD4 + T cell immune responses. Some key HLA class I (B*57:01, B*35:03) and class II (DQB1*06:02, DQB1*03:03, DB1*07:01 and DRB1:15:01) alleles have also shown confirmed associations with inter-individual variations in measles antibody responses after two doses of measles vaccine [101]. Furthermore, in a large cohort of 2,506 healthy immunized subjects (age 11 to 41 years), specific class I and class II HLA types – such as B*57:01, DQB1*06:02, and DRB1*15:05 – have been clearly associated with measles vaccine-specific neutralizing antibody titers [102]. These reproducible associations between HLA molecules and immune response outcomes have led to the identification of measles virus epitopes presented by HLA [103–105]. Such synthetic peptides/epitopes can be used to design personalized measles vaccines [106,107].

Multiple population-based vaccine studies have also demonstrated associations between candidate genes/SNPs and variations in measles vaccine-induced immune responses, including vaccine non-response and vaccine failure [17,26,101,106,108–124]. One study included healthy children (n = 745) who received two doses of measles vaccine and were genotyped for a panel of innate SNP markers, such as vitamin A (RARA, RARB, and RARG), and vitamin

D receptor (RXRA) genes, a transmembrane receptor CD46, CD209 (DC-SIGN), host antiviral sensor and effector (VISA, DDX58, OAS1–3, MX2, ADAR), TRIM (TRIM 5, 22, 25) and TLR (TLR2,3,4,7,8) genes. Multiple polymorphisms and haplotypes in these genes have been found to be significantly associated with humoral and/or cellular immune response markers [109,112–114,125], and some were subsequently replicated (e.g., the *CD46* rs2724384 genetic variant [126,127]). These findings point to additional non-HLA genetic variants/genes as being critical determinants modulating the adaptive immune responses to measles vaccine. Likewise, multigenic effects on measles vaccine-induced immunity have been examined using a large collection of SNPs (n = 1,912) that tag 126 candidate genes. Combined analyses of all these SNPs provided evidence that a multigenic model may explain variations in antibody levels (p = 0.05) and in cell-mediated IFN-g ELISPOT response (p = 0.02) variance [110]. The genetic studies reported above have identified important and informative genetic determinants of measles vaccine response heterogeneity and led to a large-scale state-of-the-art genome-wide association study (GWAS) that allowed for identification of additional genetic determinants (SNPs and immune response pathways) of measles vaccine-induced immunity [126,128].

4. Genome-wide genetic association studies and measles vaccination: from genetic association to function of genetic determinants of humoral immunity

The advances in technology and statistical analysis during the last decade have allowed for enhanced genome-wide interrogation of the human genome (i.e., GWAS studies) for identification of determinants of the host response to measles vaccination in an unbiased (by prior knowledge) way [126,129–131].

In a sophisticated GWAS in children, Feenstra et al. [129] identified two genetic loci on chromosome 1 that were associated with febrile seizures after measles-mumps-rubella (MMR) vaccination but not with unrelated febrile seizures. These two genetic loci harbor the interferon-stimulated gene *IFI44L* and the measles virus receptor gene (for attenuated MV strains) *CD46*.

In a population-based study of 2,872 healthy subjects (age 11–41 years) who had received two doses of MMR vaccine, we performed measles neutralizing antibody titer assays after a second vaccination (median 3.4 years). After correcting for multiple confounding variables (e.g., age, time since last MMR vaccination, etc.) and excluding subjects with conditions affecting immune response, we documented a wide range of MV-specific humoral immunity, with a median Ab titer of 845 mIU/mL (IQR: 394 to 1,683). Among these subjects, 94 subjects (3.3%) had non-protective levels of circulating neutralizing antibody (<120 mIU/mL), and 338 subjects (11.8%) had neutralizing antibody <210 mIU/mL, which corresponds to a PRN neutralizing dose/ND_{50} titer of 120 [132]. These data and other recent reports from the literature [22,51,52,133] raise

questions about suboptimal measles vaccine-induced humoral immunity and/or waning immunity among highly vaccinated populations in a low measles exposure environment.

The above cohort was used to assess for genetic factors contributing to MV-specific neutralizing antibody response in the first GWAS study of measles vaccine-induced immunity, which estimated the heritability of vaccine-induced measles antibody titers to be ~49% [126,127]. This GWAS (unrelated to the GWAS by Feenstra B. et al., on febrile seizures [129]) independently identified the two chromosome 1 genetic regions (the 1q31.1 region harboring the *IFI44L* gene with nine significant SNPs, and the 1q32 region harboring the *CD46* gene with 20 significant non-coding SNPs) to be associated with the measured MV-specific neutralizing antibody titer after MMR vaccination. Several overlapping SNP associations were found between the two studies (i.e., the intergenic SNP rs1318653 near the *CD46* locus and the coding *IFI44L* His73Arg SNP 273,259) in concert with multiple SNP associations with immune responses not reported previously [126]. The top SNP association in subjects of European ancestry is an intronic genetic variant rs2724374 (p-value = 4.88x10^{-09}), located near the *CD46* intron 8-exon 8 boundary, which is demonstrated to affect/cause genetic splicing (the skipping of the *CD46* serine/threonine/proline-rich/STP B exon), resulting in differential abundance of CD46 isoforms associated with different genotypes [126,134,135]. The minor allele of this SNP (G) was significantly associated in a dose-response dependent manner with ~50% reduction in MV neutralizing antibody titer after vaccination [126]. This allele is likely responsible for the splicing of exon B to favor the generation of CD46 isoforms with a shorter (and less O-glycosylated) STP region, as demonstrated by a genotype-specific RT-PCR isoform analysis and a DEXSeq analysis of NGS data [126]. We and others have suggested that MV binding and fusion capacity in cells expressing the *CD46* C1/C2 (shorter) isoforms vs. the BC1/BC2 (longer) isoforms differ, and this variance may translate to differences in both viral replication and triggering of immune response pathways after live virus vaccination [126,136–138]. The functional effects of the *CD46* rs2724374 genetic variant are summarized in Figure 1. Ongoing functional studies in human cells expressing/overexpressing different CD46 isoforms demonstrate a clear difference in MV infection/replication and innate immune pathway activation depending on the prevalence of specific CD46 isoforms (*Haralambieva and Poland*, unpublished data). Lastly, the presence/absence of STP exons (associated also with difference in the O-glycosylation) in the extracellular portion of CD46 can result in altered processing, altered CD46 shedding/cleavage by metalloproteinases, altered downregulation, and cell surface expression, and profound differences in T cell function and TCR signaling [139,140].

The discovery of *CD46* and *IFI44L* genetic variants as determinants of measles vaccine-induced humoral immunity (and adverse events) after measles vaccination could translate into the development of inexpensive chips/platforms for prospective identification of potential non-responders and susceptible individuals who will eventually need additional vaccine doses, as well as improved vaccines capable of overcoming any genetic restrictions.
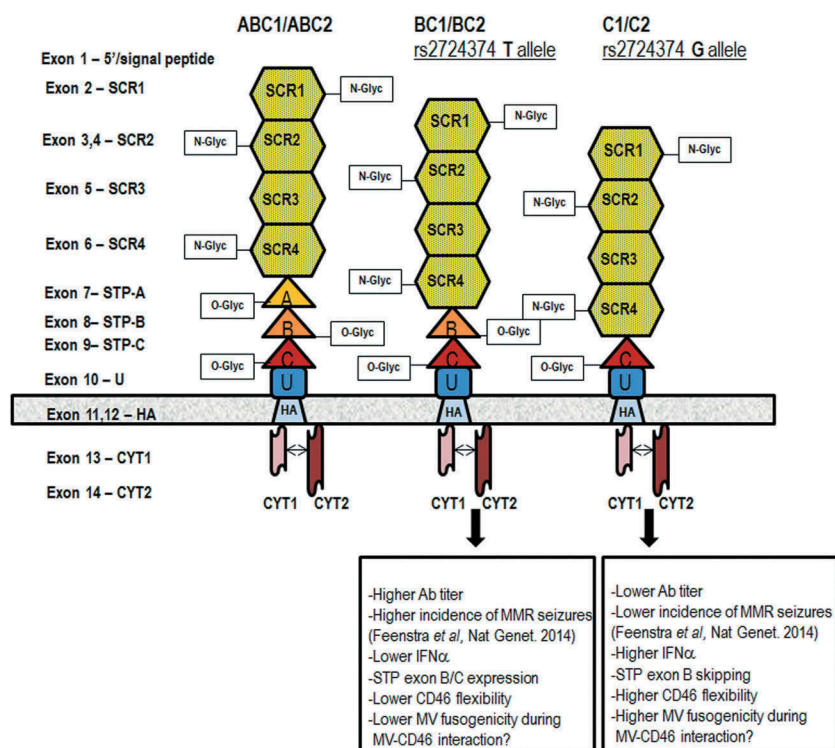
Figure 1. Measles virus receptor CD46 and functional effects of *CD46* rs2724374.

The figure is published with permission from Human Genetics [126,127]. The extracellular portion of CD46 consists of four N-glycosylated conserved short consensus repeats SCR1–4; a STP region that is O-glycosylated (encoded by exons 7/A, 8/B, and 9/C); and a region of unknown function (U). The four most common CD46 isoforms are defined based on the present STP exon/exons and the cytoplasmic tail (CYT1 or CYT2): BC1 and BC2 (with B and C exons/domains in the STP and with either CYT1 or CYT2), and C1 and C2 (with C exon/domain in the STP and with either CYT1 or CYT2). The effect of *CD46* rs2724374 on CD46 isoform prevalence (exon B expression or skipping), interaction between CD46 and MV, and immune response following measles vaccination is also summarized for the different genotypes.

5. Statistical challenges and solutions for analyzing high-dimensional data (genetic association studies, GWAS)

It is well-known that immune response is strongly influenced by both genetic and environmental factors. Because the immune system is fine-tuned [141], individual genetic factors tend to have small effects on immune response, making it statistically challenging to uncover the main causes. To overcome this challenge, careful study design and large sample sizes are crucial. The design of studies should control for factors known to influence response, such as age at immunization, sex, prior vaccinations, etc. It is also critical to control for laboratory batch effects of assay measurements and to normalize response to make unbiased comparisons [142]. Although there are multiple genes known to influence immune response, such as genes in the HLA region, the agnostic approach of GWAS offers the advantage of new discoveries, albeit at the requirement of large sample sizes.

GWAS studies [143] have achieved enormous success at identifying the genomic regions that harbor genetic determinants of complex traits [144–150], with over 2,000 traits registered in the Catalog of published genome-wide association studies with association *p*-values less than 10^{-5} with single nucleotide polymorphisms [151]. This tremendous success can be attributed to the large sample sizes required to have sufficient power to detect small effects of genes, as well as inexpensive genotyping microarrays that contain a large number of SNPs. Furthermore, large-scale reference panels provide a way to reliably impute SNPs that are close to the SNPs that are measured in microarrays [152], resulting in approximately 10 million SNPs frequently available for analyses.

To summarize GWAS *p*-values that measure the marginal association of one SNP at a time with a trait, Manhattan plots of *p*-values are frequently used, followed with LocusZoom plots for regions of interest [153]. This provides a way to focus on the SNPs with the smallest (i.e., most significant) *p*-values in distinct regions. The SNPs with the smallest *p*-values are sometimes called the lead or index SNPs. Because there are many SNPs tested for their association with a trait, GWAS results are most reliable when SNP associations achieve the accepted genome-wide statistical significance threshold of *p*-value$<5 \times 10^{-8}$ [154,155].

A caution about the lead SNP is that there is a reasonable chance that it does not have a direct causal effect on the trait [156]. Rather, the lead SNP is often in linkage disequilibrium/LD with an unmeasured causal variant [157]. This is because the SNPs on microarrays, called tag-SNPs, are chosen because they serve as surrogates for large genomic regions. Their ability to be faithful surrogates stems from their high correlation with neighboring unmeasured SNPs (i.e., high LD) [158,159]. This means that the association between a tag-SNP and a trait can be indirect, resulting from a tag-SNP statistically associated with an unmeasured causal SNP and the causal SNP having a direct effect on the

trait. To increase the density of SNPs, hopefully capturing the causal variant or at least refining its location, statistical imputation of neighboring SNPs is a widely accepted technique [160,161].

The patterns of LD among SNPs can be complex. This makes it challenging to determine the underlying causal variants by inspecting the marginal association of a trait with one SNP at a time. Statistical methods that jointly analyze all the SNPs in a region, particularly methods that are designed for fine-mapping [162,163], are useful to prioritize SNPs for subsequent functional studies. Additional insights can be gained by genomic annotation of SNPs that assign biological function based on publicly available resources [164–168].

Many of the trait-associated SNPs discovered by GWAS are not in gene-coding regions; rather, they map to non-coding regions, often in areas enriched for regulatory elements, such as enhancers, promoters, insulators, and silencers [169]. This suggests that SNPs discovered by GWAS influence the amount of expression of nearby genes (referred to as expression quantitative trait loci; eQTL), and this altered expression ultimately influences the trait. Statistical methods can be useful to integrate eQTL data (i.e., genes whose RNA levels are associated with specific SNPs) with GWAS data (i.e., traits associated with specific SNPs) in order to quantify the evidence of a causal pathway from SNP to gene-expression to a complex trait. Some methods are based on testing causal models [170], some are based on Mendelian randomization [171], and some are Bayesian approaches [172,173], but they all have the common strategy of evaluating whether mRNA is a mediator between a SNP and a trait. A variety of approaches are provided elsewhere [162].

6. Gene expression and systems biology-based approaches for the discovery of determinants/ signatures of vaccine-induced immunity

The application of high-dimensional gene expression and/or other omics technologies to reliably identify the determinants/signatures reflecting the development and maintenance of measles vaccine immunity is still in its infancy. Several gene expression studies during the course of measles infection or after measles vaccination and/or *in vitro* infection, have provided useful but limited information about the role of specific genes and pathways in the development of measles immunity [62,174,175]. Two recent mRNA-Seq studies profiling gene and miRNA expression in the cells of high and low antibody measles vaccine responders have identified essential plasma cell survival and homing factors (e.g., CD93, a key factor for the preservation of plasma cells in the bone marrow and for sustained production of antibodies [176]) and B cell-specific miRNA expression patterns [177] that were associated with neutralizing antibody titer after vaccination.

Systems biology approaches have also yielded important insights into the development of humoral immunity following vaccination. Most systems vaccinology studies focus on the high dimensional analysis of the transcriptome, proteome, metabolome, and/or microbiome. It is equally important to comprehensively evaluate the immune response both in

terms of the cell subsets involved and the effector functions produced. This type of approach can be especially useful for pathogens without clearly defined correlates of protection [71], as humoral immunity can be conferred by antigen-specific antibodies with myriad effector functions including: neutralization, complement fixation, opsonization, and enhancement of cellular responses. Each of these effector functions can then be linked to specific gene expression patterns, or 'signatures,' necessary for their development. Querec et al. were able to identify a gene signature that predicted neutralizing antibody responses to the yellow fever vaccine with 100% accuracy [178]. This signature involved expression of *TNFRSF17*, a gene producing the receptor for the BLyS-BAFF B cell growth factor. A similar study across three influenza seasons found that expression levels of *CaMKIV* were inversely proportional to the titer of hemagglutination-inhibiting antibodies [179]. Genomic signatures have also been found to be associated with protection following vaccination with the RTS, S malaria vaccine [180]. Collectively, these and similar studies identify critical pathways necessary for the development of humoral immunity. Further research is needed to identify how these and/or other genes/pathways contribute to measles immunity. The resulting signatures may serve as important predictive biomarkers of immunogenicity or vaccine efficacy.

Novel technologies are allowing researchers to investigate immune responses at an unprecedented level of detail. Mass cytometry (CyTOF®) using heavy-metal conjugated antibodies has expanded the number of parameters one can measure from 12–18 to 40+. This increase in capability enables more comprehensive immune profiling of leukocyte phenotype and higher resolution of functional characteristics (Figure 2). Similarly, single-cell sequencing (scRNA-Seq) allows us to evaluate individual cellular transcriptomes rather than the average gene expression of all cells in a biological specimen (Figure 2). One can observe the expression level of transcription factors, cellular receptors, signaling molecules, and other immunologically important genes within relevant cell types [181]. Sequencing of individual T cell and B cell receptors provides information on T/B cell diversity, clonal expansion, and somatic hypermutation [182]. An exciting next step in the field of systems vaccinology will be the combination of multiple single-cell resolution technologies into multi-omics approaches [183]. One example is CITE-Seq (or REAP-Seq), which can be used to simultaneously characterize both surface expression and transcriptome in individual cells, thereby combining the advantages of next-generation sequencing and flow cytometry. One might use these techniques to assess the individual transcriptomes of tetramer-positive cells in order to gain insights into transcriptional activity in antigen-specific cells [184,185]. Similarly, scMT-Seq or scTrio-Seq allow for the simultaneous analysis of the genome, transcriptome, and epigenome of individual cells for a comprehensive cell-specific snapshot of genetic landscape and activity [186,187].

7. Expert commentary

Given the morbidity and mortality of measles, repeated importations, continuing outbreaks, contraindications to live attenuated measles vaccines for an increasingly
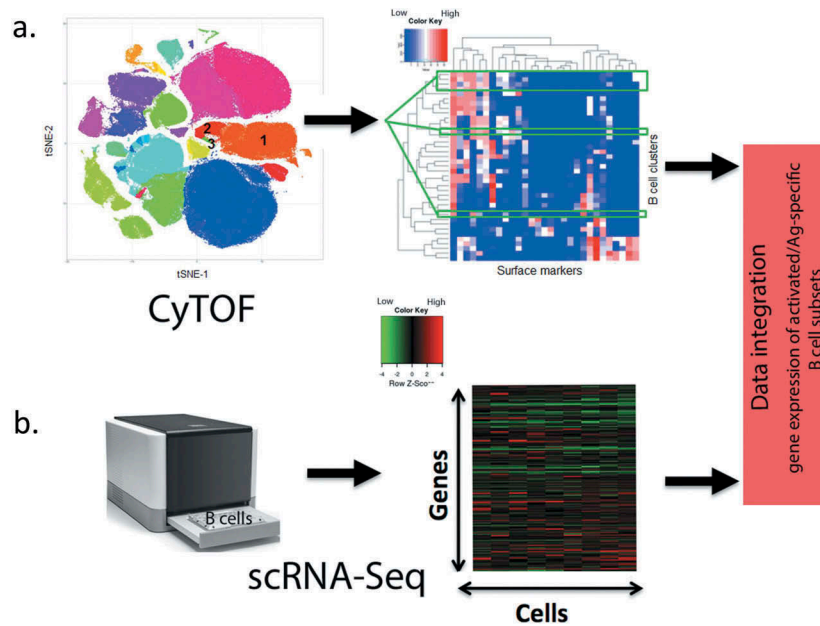
Figure 2. CyTOF and scRNA-Seq analysis of B cell subsets after vaccination. a) Schematic representation of CyTOF. t-SNE plot of cell clusters defined by cellular markers. The annotation of the numbered cell clusters is as follows: 1. naïve B cells; 2. memory B and 3. plasmablasts. For clarity, only three of the relevant B cell clusters are shown. Heat map displaying the expression levels (blue = low, red = high) of each cell surface marker in columns and each B cell cluster of interest in rows. b) Schematic representation of scRNA-Seq. Heat map displaying the gene expression levels (green = low, red = high) within single B cells (assay cell input is purified B cells). Data integration allows for the identification of gene expression signatures within activated and/or antigen-specific B cell subsets after vaccination.

immunocompromised population, and unmerited concerns over the safety of the current measles vaccine, it is apparent that new vaccine types are needed.

Unique among vaccine-preventable human pathogens, measles is the most transmissible human disease – requiring at least 90–95% herd immunity for disease control. The current vaccine licensed in the United States has a measurable primary and secondary failure rate that leads to population-level immunity that is often less than that required for herd immunity [17,49]. This is especially true in regions of the world where seroconversion rates for MMR (and other measles-containing vaccines) are lower than in the United States and Europe.

The ideal measles vaccine, even if parenteral, should induce lifelong immunity after one dose, have little or no contra-indications, be manufactured inexpensively, not require a cold chain and be safely stored for long periods of time, and be administered by a variety of health-care personnel – particularly in low-income countries.

For many of the above reasons, even more, appealing would be vaccines that do not require parenteral administration and could be given to infants below the age of 12 months (i.e., no maternal antibody interference), ideally without the need for highly trained health-care personnel and suitable for use in low resource settings. In this regard, oral or skin patch vaccines would be ideal candidates if they could be inexpensively made.

To this end, possibilities currently being investigated include protein and peptide-based vaccines using one or more measles viral proteins (H, F, and N proteins and peptides), recombinant protein vaccines, DNA-based vaccine constructs, and virus-vectored vaccines, as well as different delivery systems, such as microneedle skin patch vaccines and aerosol vaccines [107,117,188–195]. A unique recently developed administration method is via oral disintegrating films, which have been loaded with measles virus nanoparticles. Early studies in pigs have been promising [196].

8. Five-year view

Over the next five years, we will continue to see an increasingly sophisticated understanding of measles vaccine-induced humoral immunity. Currently, humoral 'measles immunity' is assessed most commonly using either EIA assays or fluorescence-based immunoassays. In research settings, measles neutralizing antibody may also be measured. The former two assays are not direct functional antibody assays and hence can be misleading. Neutralizing antibody, on the other hand, is a direct measure of functional antibody responses, that has been correlated with protection against disease, and therefore, is a better measure of humoral immunity. However, the assay is laborious, expensive, and has a lower throughput; therefore, it is not generally used clinically. In addition, our current correlate of immunity/protection is incomplete, as it does not evaluate cellular immune or other parameters that may also be important for durable and/or protective immunity. Systems biology studies are beginning to reveal genetic and molecular 'signatures' of the protective immune response. In time, it may be possible to narrow such signatures to highly predictive assays of vaccine responsiveness and efficacy/effectiveness, and to identify precise correlates of protection. In addition, protein and antibody array assays may provide a more holistic view

of humoral immune response; in other words, they will allow direct measures of antibody response to specific proteins within a pathogen. In the case of measles, for example, we may be able to measure anti-H, anti-F, anti-N, and anti-P antibodies or specific antibody patterns as better correlates of immunity in a high-throughput, low-cost manner. Finally, measures of immune durability are critically needed, and much research is warranted on this topic.

Key issues

- Measles outbreaks continue to occur in high-vaccine coverage and low-measles exposure settings.
- To address the barriers to measles elimination activities and the current knowledge gap, new/additional correlates of protection and new approaches for evaluating and predicting humoral immune response after measles vaccination are needed.
- Genetic determinants of measles vaccine-induced neutralizing antibody response (e.g., *CD46* and *IFI44L* genetic variants, other genetic markers) are under investigation.
- Systems biology and/or other 'omics' studies are likely to identify 'signatures' of the protective and durable immune response after measles vaccination.
- The discovery of predictive 'signatures' of measles vaccine immunogenicity, efficacy, and long-term effectiveness will identify individuals in need of additional vaccine doses and/or new improved measles vaccines.

Disclosure of whether any or part of the data has been previously published

Figure 1 in this review has been previously published (Hum Genet. 2017;136:421–435, PMID: 28289848) [126, 127] and is used with permission from *Human Genetics*.

Declaration of interest

G.A. Poland is the chair of a Safety Evaluation Committee for novel investigational vaccine trials being conducted by Merck Research Laboratories. G.A. Poland offers consultative advice on vaccine development to Merck & Co. Inc., CSL Biotherapies, Avianax, Dynavax, Novartis Vaccines and Therapeutics, Emergent Biosolutions, Adjuvance, Microdermis, Seqirus, NewLink, Protein Sciences, GSK Vaccines, and Sanofi Pasteur. He holds three patents related to measles and vaccinia peptide research. R.B. Kennedy has received funding from Merck Research Laboratories to study waning immunity to measles and mumps after immunization with MMR-II. I.G. Ovsyannikova holds three patents related to measles and vaccinia peptide research. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

Reviewer disclosures

Peer reviewers on this manuscript have no relevant financial or other relationships to disclose.

References

Papers of special note have been highlighted as either of interest (•) or of considerable interest (••) to readers.

1. World Health Organization. Progress towards regional measles elimination – worldwide, 2000–2016. Releve epidemiologique hebdomadaire. 2017 Oct 27;92(43):649–659.
2. Dabbagh A, Patel MK, Dumolard L, et al. Progress toward regional measles elimination - worldwide, 2000–2016. MMWR. 2017 Oct 27;66(42):1148–1153.
3. Centers for Disease Control and Prevention. Measles Cases and Outbreaks.[cited 2018 Sep 30] Available from: http://www.cdc.gov/measles/cases-outbreaks.html
4. Hall V, Banerjee E, Kenyon C, et al. Measles outbreak - Minnesota April-May 2017. MMWR. 2017 Jul 14;66(27):713–717.
5. World Health Organization. Europe observes a 4-fold increase in measles cases in 2017 compared to previous year.[cited 2018 Sept 30]. Available from: http://www.euro.who.int/en/media-centre/sections/press-releases/2018/europe-observes-a-4-fold-increase-in-measles-cases-in-2017-compared-to-previous-year
6. Williams S European measles cases quadrupled in 2017. The Scientist. 2018 Available from: https://www.the-scientist.com/the-nutshell/european-measles-cases-quadrupled-in-2017-30261
7. Mina MJ, Metcalf CJ, de Swart RL, et al. Long-term measles-induced immunomodulation increases overall childhood infectious disease mortality. Science. 2015 May 8;348(6235):694–699.
   • Provides statistical evidence for the non-specific effects of measles on host resistance/immunity to other pathogens.
8. de Vries RD, McQuaid S, van AG, et al. Measles immune suppression: lessons from the macaque model. PLoS Pathog. 2012;8(8): E1002885.
   •• Explains measles-induced immunosuppression from infection and immune-mediated lymphocyte depletion of follicular B and CD45RA(-) memory T-lymphocytes.
9. de Vries RD, de Swart RL. Measles immune suppression: functional impairment or numbers game? PLoS Pathog. 2014 Dec;10(12): E1004482.
   •• Explains measles-induced immune suppression resulting from depletion of immune cell subsets, which is masked by the proliferation of MV-specific and bystander lymphocytes (explains the "measles paradox").
10. Laksono BM, Grosserichter-Wagener C, de Vries RD, et al. In vitro measles virus infection of human lymphocyte subsets demonstrates high susceptibility and permissiveness of both naive and memory B cells. J Virol. 2018 Apr 15;92:8.
    • Demonstrates the high susceptibility/permissiveness of human memory B cells to measles virus.
11. Laksono BM, de Vries RD, McQuaid S, et al. Measles virus host invasion and pathogenesis. Viruses. 2016 Jul 28;8:8.
12. Wendorf KA, Winter K, Zipprich J, et al. Subacute sclerosing panencephalitis: the devastating measles complication that might be more common than previously estimated. Clinl Infect Dis. 2017 Jul 15;65(2):226–232.
13. Muller CP, Kremer JR, Best JM, et al. Reducing global disease burden of measles and rubella: report of the WHO Steering Committee on research related to measles and rubella vaccines and vaccination, 2005. Vaccine. 2007;25(1):1–9.
14. Patel MK, Gacic-Dobo M, Strebel PM, et al. Progress toward regional measles elimination - worldwide, 2000–2015. MMWR. 2016 Nov 11;65(44):1228–1233.
15. Meissner HC, Strebel PM, Orenstein WA. Measles vaccines and the potential for worldwide eradication of measles. Pediatrics. 2004;114(4):1065–1069.
16. Elliman D, Sengupta N. Measles. Curr Opin Infect Dis. 2005;18 (3):229–234.

17. Haralambieva IH, Ovsyannikova IG, Pankratz VS, et al. The genetic basis for interindividual immune response variation to measles vaccine: new understanding and new vaccine approaches. Expert Rev Vaccines. 2013 Jan;12(1):57–70.
   • **Summarizes the immunogenetic findings underlying the inter-individual immune response variation to measles vaccine.**
18. Uzicanin A, Zimmerman L. Field effectiveness of live attenuated measles-containing vaccines: a review of published literature. J Infect Dis. 2011;204(Suppl 1):S133–S148.
19. Boulianne N, De Serres G, Ratnam S, et al. Measles, mumps, and rubella antibodies in children 5–6 years after immunization: effect of vaccine type and age at vaccination. Vaccine. 1995;13:1611–1616.
20. Christenson B, Bttiger M. Measles antibody: comparison of long-term vaccination titres, early vaccination titres and naturally acquired immunity to and booster effects on the measles virus. Vaccine. 1994;12:129–133.
21. Mitchell LA, Tingle AJ, Décarie D, et al. Serologic responses to measles, mumps, and rubella (MMR) vaccine in healthy infants: failure to respond to measles and mumps components may influence decisions on timing of the second dose of MMR. Can J Public Health. 1998;89(5):325–328.
22. Moss W. Measles in vaccinated individuals and the future of measles elimination. Clin Infect Dis. 2018;67:1320–1321.
23. Moss WJ. Measles control and the prospect of eradication. Curr Top Microbiol Immunol. 2009;330:173–189.
24. Measles Fact Sheet No. 286. Available from: http://www.who.int/en/news-room/fact-sheets/detail/measles. 2018.
25. Parker Fiebelkorn A, Redd SB, Gallagher K, et al. Measles in the United States during the postelimination era. J Infect Dis. 2010;202 (10):1520–1528.
26. Ga P, Rm J, Am T, et al. Measles re-immunization in children seronegative after initial immunization. JAMA. 1997;277:1156–1158.
27. Seward JF, Orenstein WA. A rare event: A measles outbreak in a population with high 2-dose measles vaccine coverage. Clinl Infect Dis. 2012;55(3):403–405.
28. De Serres G, Boulianne N, Defay F, et al. Higher risk of measles when the first dose of a 2-dose schedule of measles vaccine is given at 12–14 months versus 15 months of age. Clin Infect Dis. 2012;55(3):394–402.
29. Ga P, Rm J. Failure to reach the goal of measles elimination. Apparent paradox of measles infections in immunized persons. Arch Internal Med. 1994;154:1815–1820.
30. Paunio M, Peltola H, Valle M, et al. Explosive school-based measles outbreak: intense exposure may have resulted in high risk, even among revaccinees. Am J Epidemiol. 1998;148(11):1103–1110.
31. Mathias RG, Meekison WG, Arcand TA, et al. The role of secondary vaccine failures in measles outbreaks. Am J Public Health. 1989;79:475–478.
32. Sugerman DE, Barskey AE, Delea MG, et al. Measles outbreak in a highly vaccinated population, San Diego, 2008: role of the intentionally undervaccinated. Pediatrics. 2010;125(4):747–755.
33. Pannuti CS, Morello RJ, Moraes JC, et al. Identification of primary and secondary measles vaccine failures by measurement of immunoglobulin G avidity in measles cases during the 1997 Sao Paulo epidemic. Clin Diagn Lab Immunol. 2004;11(1):119–122.
34. Hickman CJ, Hyde TB, Sowers SB, et al. Laboratory characterization of measles virus infection in previously vaccinated and unvaccinated individuals. J Infect Dis. 2011;204(Suppl 1):S549–S558.
35. Glass K, Grenfell BT. Waning immunity and subclinical measles infections in England. Vaccine. 2004;22(29–30):4110–4116.
36. Mossong J, Nokes DJ, Edmunds WJ, et al. Modeling the impact of subclinical measles transmission in vaccinated populations with waning immunity. Am J Epidemiol. 1999;150(11):1238–1249.
37. Ja W, Ga P. Measles and mumps outbreaks in the United States: think globally, vaccinate locally [editorial]. Vaccine. 2014 Aug 20;32 (37):4703–4704.
38. Papania MJ, Wallace GS, Rota PA, et al. Elimination of endemic measles, rubella, and congenital rubella syndrome from the Western hemisphere: the US experience [historical article]. JAMA Pediatr. 2014 Feb;168(2):148–155.
39. Centers for Disease Control and Prevention. Notes from the field: measles outbreak associated with a traveler returning from India - North Carolina, april-may 2013. MMWR. 2013 Sep 13;62(36):753.
40. Centers for Disease Control and Prevention. Notes from the field: measles outbreak among members of a religious community - Brooklyn, New York, March-June 2013. MMWR 2013 Sep 13;62 (36):752–753.
41. Zipprich J, Hacker JK, Murray EL, et al. Notes from the field: measles - California, January 1-april 18, 2014. MMWR. 2014 Apr 25;63 (16):362–363.
42. Centers for Disease Control and Prevention. Notes from the field: measles outbreak–Indiana, June-July 2011. Morbidity and Mortality Weekly Report. 2011;34:1169.
43. Notes from the field: Multiple cases of measles after exposure during air travel–Australia and New Zealand, January. 2011. MMWR. 2011;60(25):851.
44. Notes from the field: measles outbreak–Hennepin county, Minnesota, February–March. 2011. MMWR. 2011;60(13):421.
45. Katz SL, Hinman AR. Summary and conclusions: measles elimination meeting, 16–17 March 2000. J Infect Dis. 2004;189(Suppl 1): S43–S47.
46. Collective Editorial Team. Measles once again endemic in the United Kingdom [News]. Euro Surveill. Jul 3 2008;13(27).
47. Centers for Disease Control and Prevention. Measles - United States, 2011. MMWR. 2012;61:253–257.
48. Rosen JB, Rota JS, Hickman CJ, et al. Outbreak of measles among persons with prior evidence of immunity, New York City, 2011. Clin Infect Dis. 2014 May;58(9):1205–1210. PubMed PMID: 24585562; eng.
49. Haralambieva IH, Kennedy RB, Ovsyannikova IG, et al. Variability in humoral immunity to measles vaccine: new developments. Trends Mol Med. 2015 Dec;21(12):789–801.
   •• **Summarizes new studies/findings underlying variability in humoral immunity to measles vaccine.**
50. LE M, Sr P, Pem F, et al. Duration of live measles vaccine-induced immunity. Pediatr Infect Dis J. 1990;9:101–110.
51. Cherry JD, Zahn M. Clinical characteristics of measles in previously vaccinated and unvaccinated patients in California. Clin Infect Dis. 2018;67(9):1315–1319.
   • **Confirms measles in subjects with ≥2 doses of measles vaccine (11% of 232 US measles cases with verified vaccination history).**
52. Clemmons NS, Wallace GS, Patel M, et al. Incidence of measles in the United States, 2001–2015. JAMA. 2017 Oct 3;318 (13):1279–1281.
53. Increased transmission and outbreaks of measles–European Region. 2011. MMWR. 2011 Dec 2;60(47):1605–1610.
54. Griffin DE. Measles Vaccine. Viral Immunol. 2018 Mar;31(2):86–95.
55. Sowers SB, Rota JS, Hickman CJ, et al. High concentrations of measles neutralizing antibodies and high-avidity measles IgG accurately identify measles reinfection cases. Clin Vaccine Immunol. 2016 Aug;23(8):707–716.
56. Hahne SJ, Nic Lochlainn LM, van Burgel ND, et al. Measles outbreak among previously immunized healthcare workers, the Netherlands, 2014. J Infect Dis. 2016 Dec 15;214(12):1980–1986.
57. Tatsuo H, Ono N, Tanaka K, et al. SLAM (CDw150) is a cellular receptor for measles virus. Nature. 2000;406(6798):893–897.
58. Noyce RS, Bondre DG, Ha MN, et al. Tumor cell marker PVRL4 (nectin 4) is an epithelial cell receptor for measles virus. PLoS Pathog. 2011;7(8):e1002240.
   •• **Discovers PVRL4 (Nectin 4) as a cell receptor for measles virus**
59. Muhlebach MD, Mateo M, Sinn PL, et al. Adherens junction protein nectin-4 is the epithelial receptor for measles virus. Nature. 2011;480(7378):530–533.
   •• **Discovers PVRL4 (Nectin 4) as a cell receptor for measles virus.**
60. Cattaneo R. Four viruses, two bacteria, and one receptor: membrane cofactor protein (CD46) as pathogens' magnet. J Virol. 2004;78(9):4385–4388.

61. Griffin DE. The immune response in measles: virus control, clearance and protective immunity. Viruses. 2016 Oct 12;8(10):86–95.

62. Zilliox MJ, Parmigiani G, Griffin DE. Gene expression patterns in dendritic cells infected with measles virus compared with other pathogens. Proc Natl Acad Sci USA. 2006;103(9):3363–3368.

63. Haralambieva IH, Ovsyannikova IG, Dhiman N, et al. Differential cellular immune responses to wild-type and attenuated edmonston tag measles virus strains are primarily defined by the viral phosphoprotein gene. J Med Virol. 2010;82(11):1966–1975.

64. Chen RT, Markowitz LE, Albrecht P, et al. Measles antibody: reevaluation of protective titers. J Infect Dis. 1990;162(5):1036–1042.

65. de Swart RL, Yuksel S, Osterhaus AD. Relative contributions of measles virus hemagglutinin- and fusion protein-specific serum antibodies to virus neutralization. J Virol. 2005;79(17):11547–11551.

66. Polack FP, Lee SH, Permar S, et al. Successful DNA immunization against measles: neutralizing antibody against either the hemagglutinin or fusion glycoprotein protects rhesus macaques without evidence of atypical measles. Nat Med. 2000;6(7):776–781.

67. Bouche FB, Ertl OT, Muller CP. Neutralizing B cell response in measles. Viral Immunol. 2002;15(3):451–471.
•• Reviews and summarises findings on mealses-specific B cell responses.

68. de Vries RD, de Swart RL. Evaluating measles vaccines: can we assess cellular immunity? Exp Rev Vaccines. 2012 Jul;11(7):779–782.

69. Crotty S. A brief history of T cell help to B cells. Nat Rev Immunol. 2015 Mar;15(3):185–189.

70. Haralambieva IH, Ovsyannikova IG, Vierkant RA, et al. Development of a novel efficient fluorescence-based plaque reduction microneutralization assay for measles immunity. Clin Vaccine Immunol. 2008;15(7):1054–1059.

71. Plotkin SA. Correlates of protection induced by vaccination. Clin Vacci ne Immunol. 2010;17(7):1055–1065.
• Summarizes correlates of protection after vaccination with different vaccines.

72. Haralambieva IH, Ovsyannikova IG, O'Byrne M, et al. 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. 2011;29(27):4485–4491.

73. Polack FP, Hoffman SJ, Crujeiras G, et al. A role for nonprotective complement-fixing antibodies with low avidity for measles virus in atypical measles. Nat Med. 2003;9(9):1209–1213.

74. World Health Organization. Correlates of vaccine-induced protection: methods and implications. Geneva, Switzerland 2013. Available from: http://apps.who.int/iris/bitstream/handle/10665/84288/WHO_IVB_13.01_eng.pdf;jsessionid=F944A52651CB43AEC79D14568617D67D?sequence=1

75. Dimech W, Mulders MN. A review of testing used in seroprevalence studies on measles and rubella. Vaccine. 2016 Jul 29;34(35):4119–4122.

76. Dimech W, Mulders MN. A 16-year review of seroprevalence studies on measles and rubella. Vaccine. 2016 Jul 29;34(35):4110–4118.

77. Thompson KM, Odahowski CL. Systematic review of measles and rubella serology studies. Risk Anal. 2016 Jul;36(7):1459–1486.

78. Binnicker MJ, Jespersen DJ, Rollins LO. Evaluation of the bio-rad bioplex measles, mumps, rubella, and varicella-zoster virus IgG multiplex bead immunoassay. Clin Vaccine Immunol. 2011 Sep;18(9):1524–1526.

79. Smits GP, van Gageldonk PG, Schouls LM, et al. Development of a bead-based multiplex immunoassay for simultaneous quantitative detection of IgG serum antibodies against measles, mumps, rubella, and varicella-zoster virus. Clin Vaccine Immunol. 2012 Mar;19(3):396–400.

80. Tischer A, Andrews N, Kafatos G, et al. Standardization of measles, mumps and rubella assays to enable comparisons of seroprevalence data across 21 European countries and Australia. Epidemiol Infect. 2007 Jul;135(5):787–797.

81. Hubschen JM, Bork SM, Brown KE, et al. Challenges of measles and rubella laboratory diagnostic in the era of elimination. Clin Microbiol Infect. 2017 Aug;23(8):511–515.

82. World Health Organization. Expert committee on Biological standardization, October 8–12, 2007: report on the collaborative study to investigate the relationship between the 1st IRP and the 2nd and 3rd international standards for anti-measles serum/plasma in both ELISA and PRNT. Geneva, Switzerland 2007. Available from: http://www.who.int/biologicals/BS07%202076anti-measles.pdf.

83. Haralambieva IH, Simon WL, Kennedy RB, et al. Profiling of measles-specific humoral immunity in individuals following two doses of MMR vaccine using proteome microarrays. Viruses. 2015;7(3):1113–1133.
• Provides data on measles-specific antibody levels to different measles virus proteins after vaccination.

84. Mercader S, Garcia P, Bellini WJ. Measles virus IgG avidity assay for use in classification of measles vaccine failure in measles elimination settings. Clin Vaccine Immunol. 2012 Nov;19(11):1810–1817.
• Provides methodology (measles-specific IgG avidity assay) for classification of measles vaccine failure in settings with high vaccine coverage.

85. Latner DR, McGrew M, Williams N, et al. Enzyme-linked immunospot assay detection of mumps-specific antibody-secreting B cells as an alternative method of laboratory diagnosis. Clin Vaccine Immunol. 2011;18(1):35–42.

86. Carter MJ, Mitchell RM, Meyer Sauteur PM, et al. The antibody-secreting cell response to infection: kinetics and clinical applications. Front Immunol. 2017;8:630.

87. Kakoulidou M, Ingelman-Sundberg H, Johansson E, et al. Kinetics of antibody and memory B cell responses after MMR immunization in children and young adults. Vaccine. 2013 Jan 11;31(4):711–717.

88. Buisman AM, de Rond CG, Ozturk K, et al. Long-term presence of memory B-cells specific for different vaccine components. Vaccine. 2009 Dec 10;28(1):179–186.

89. Titanji K, De Milito A, Cagigi A, et al. Loss of memory B cells impairs maintenance of long-term serologic memory during HIV-1 infection. Blood. 2006 Sep 1;108(5):1580–1587.

90. Ellebedy AH, Jackson KJ, Kissick HT, et al. Defining antigen-specific plasmablast and memory B cell subsets in human blood after viral infection or vaccination. Nat Immunol. 2016 Oct;17(10):1226–1234.

91. Hammarlund E, Thomas A, Amanna IJ, et al. Plasma cell survival in the absence of B cell memory. Nat Commun. 2017 Nov 24;8(1):1781.
•• Provides evidence that long-lived plasma cells represent a key cell population responsible for long-term antibody production.

92. Amanna IJ, Carlson NE, Slifka MK. Duration of humoral immunity to common viral and vaccine antigens. N Engl J Med. 2007;357(19):1903–1915.

93. Amanna IJ, Slifka MK. Mechanisms that determine plasma cell lifespan and the duration of humoral immunity. Immunol Rev. 2010 Jul;236:125–138.
•• Reviews findings and hypotheses on plasma cells with regard to the duration of humoral immunity (antibody response).

94. Fairfax KA, Kallies A, Nutt SL, et al. Plasma cell development: from B-cell subsets to long-term survival niches. Semin Immunol. 2008 Feb;20(1):49–58.

95. Radbruch A, Muehlinghaus G, Luger EO, et al. Competence and competition: the challenge of becoming a long-lived plasma cell. Nat Rev Immunol. 2006 Oct;6(10):741–750.
•• Reviews findings on long-lived plasma cells with regard to the duration of humoral immunity (antibody response).

96. Pensieroso S, Cagigi A, Palma P, et al. Timing of HAART defines the integrity of memory B cells and the longevity of humoral responses in HIV-1 vertically-infected children. Proc Natl Acad Sci USA. 2009 May 12;106(19):7939–7944.

97. Rocca S, Santilli V, Cotugno N, et al. Waning of vaccine-induced immunity to measles in kidney transplanted children. Medicine (Baltimore). 2016 Sep;95(37):e4738.

98. Kotton CN. Immunization after kidney transplantation-what is necessary and what is safe? Nat Rev Nephrol. 2014 Oct;10(10):555–562.

99. Cotugno N, Finocchi A, Cagigi A, et al. Defective B-cell proliferation and maintenance of long-term memory in patients with chronic granulomatous disease. J Allergy Clin Immunol. 2015 Mar;135(3):753–61 e2.

100. World Health Organization. Global measles & rubella strategic plan 2012–2020. Geneva 2012. http://apps.who.int/iris/bitstream/10665/44855/1/9789241503396_eng.pdf.

101. Ovsyannikova IG, Pankratz VS, Vierkant RA, et al. Consistency of HLA associations between two independent measles vaccine cohorts: a replication study. Vaccine. 2012;30(12):2146–2152.

102. Ovsyannikova IG, Schaid DJ, Larrabee BR, et al. A large population-based association study between HLA and KIR genotypes and measles vaccine antibody responses. PLoS One. 2017;12(2):e0171261.

103. Schellens IM, Meiring HD, Hoof I, et al. Measles virus epitope presentation by HLA: novel insights into epitope selection, dominance, and microvariation. Front Immunol. 2015;6:546.

104. Ovsyannikova IG, Johnson KL, Muddiman DC, et al. Identification and characterization of novel, naturally processed measles virus class II HLA-DRB1 peptides. J Virol. 2004;78(1):42–51.

105. Ovsyannikova IG, Johnson KL, Naylor S, et al. Naturally processed measles virus peptide eluted from class II HLA-DRB1*03 recognized by T lymphocytes from human blood. Virology. 2003;312 (2):495–506.

106. Ga P, Ig O, Rb K, et al. Vaccinomics and a new paradigm for the development of preventive vaccines against viral infections. Omics. 2011;15(9):625–636.

107. Ovsyannikova IG, Johnson KL, Bergen HR III, et al. Mass spectrometry and peptide-based vaccine development. Clin Pharmacol Ther. 2007;82(6):644–652.

108. Clifford HD, Hayden CM, Khoo SK, et al. Polymorphisms in key innate immune genes and their effects on measles vaccine responses and vaccine failure in children from Mozambique. Vaccine. 2012 Sep 21;30(43):6180–6185.

109. Ovsyannikova IG, Haralambieva IH, Vierkant RA, et al. The role of polymorphisms in toll-like receptors and their associated intracellular signaling genes in measles vaccine immunity. Hum Genet. 2011;130(4):547–561.

110. Kennedy RB, Ovsyannikova IG, Haralambieva IH, et al. Multigenic control of measles vaccine immunity mediated by polymorphisms in measles receptor, innate pathway, and cytokine genes. Vaccine. 2012;30(12):2159–2167.

111. Ga P, Ig O, Rm J, et al. Heterogeneity in vaccine immune response: the role of immunogenetics and the emerging field of vaccinomics. Clin Pharmacol Ther. 2007;82(6):653–664.

112. Ovsyannikova IG, Haralambieva IH, Vierkant RA, et al. Effects of vitamin A and D receptor gene polymorphisms/haplotypes on immune responses to measles vaccine. Pharmacogenet Genomics. 2012;22(1):20–31.

113. Ovsyannikova IG, Haralambieva IH, Vierkant RA, et al. The association of CD46, SLAM, and CD209 cellular receptor gene SNPs with variations in measles vaccine-induced immune responses–a replication study and examination of novel polymorphisms. Hum Hered. 2011;72(3):206–223.

114. Haralambieva IH, Ovsyannikova IG, Umlauf BJ, et al. Genetic polymorphisms in host antiviral genes: associations with humoral and cellular immunity to measles vaccine. Vaccine. 2011;29 (48):8988–8997.

115. Haralambieva IH, Ovsyannikova IG, Kennedy RB, et al. Associations between single nucleotide polymorphisms and haplotypes in cytokine and cytokine receptor genes and immunity to measles vaccination. Vaccine. 2011;29(45):7883–7895. PubMed Central PMCID: PMCPMCID: PMC3191314.

116. Yoo KH, Agarwal K, Butterfield M, et al. Assessment of humoral and cell-mediated immune response to measles-mumps-rubella vaccine viruses among patients with asthma. Allergy Asthma Proc. 2010;31(6):499–506.

117. Ga P, Ig O, Rm J. Application of pharmacogenomics to vaccines. Pharmacogenomics. 2009;10(5):837–852.

118. Dhiman N, Ovsyannikova IG, Vierkant RA, et al. Associations between SNPs in toll-like receptors and related intracellular signaling molecules and immune responses to measles vaccine: preliminary results. Vaccine. 2008;26(14):1731–1736.

119. Ga P, Ig O, Rm J. Genetics and immune response to vaccines. In: Ra K, Jm M, Avs H, editors. Genetic susceptibility to infectious diseases. New York: Oxford University Press; 2008. p. 1–447.

120. Clifford HD, Yerkovich ST, Khoo SK, et al. TLR3 and RIG-I gene variants: associations with functional effects on receptor expression and responses to measles virus and vaccine in vaccinated infants. Hum Immunol. 2012;73(6):677–685.

121. Clifford HD, Hayden CM, Khoo SK, et al. CD46 measles virus receptor polymorphisms influence receptor protein expression and primary measles vaccine responses in naive Australian children. Clin Vaccine Immunol. 2012;19(5):704–710.

122. Clifford HD, Yerkovich ST, Khoo SK, et al. Toll-like receptor 7 and 8 polymorphisms: associations with functional effects and cellular and antibody responses to measles virus and vaccine. Immunogenetics. 2012;64(3):219–228.

123. Clifford HD, Richmond P, Khoo SK, et al. SLAM and DC-SIGN measles receptor polymorphisms and their impact on antibody and cytokine responses to measles vaccine. Vaccine. 2011;29 (33):5407–5413.

124. Newport MJ. The genetic regulation of infant immune responses to vaccination. Front Immunol. 2015;6:18.

125. Ovsyannikova IG, Haralambieva IH, Vierkant RA, et al. Associations between polymorphisms in the antiviral TRIM genes and measles vaccine immunity. Hum Immunol. 2013 Jun;74(6):768–774.

126. Haralambieva IH, Ovsyannikova IG, Kennedy RB, et al. Genome-wide associations of CD46 and IFI44L genetic variants with neutralizing antibody response to measles vaccine. Hum Genet. 2017;136(4):421–435.
•• Discovers CD46 and IFI44L genetic variants as important determinants of neutralizing antibody response to measles vaccination.

127. Schaid DJ, Haralambieva IH, Larrabee BR, et al. Heritability of vaccine-induced measles neutralizing antibody titers. Vaccine. 2017;35(10):1390–1394.

128. Marchini J, Donnelly P, Cardon LR. Genome-wide strategies for detecting multiple loci that influence complex diseases. Nat Genet. 2005;37(4):413–417.

129. Feenstra B, Pasternak B, Geller F, et al. Common variants associated with general and MMR vaccine-related febrile seizures. Nat Genet. 2014 Dec;46(12):1274–1282.
•• Discovers genetic drivers of MMR vaccine-associated febrile seizures.

130. Pasternak B, Feenstra B, Melbye M, et al. Improving vaccine safety through a better understanding of vaccine adverse events. Clin Infect Dis. 2015 May 15;60(10):1586–1587.

131. Hammer C, Begemann M, McLaren PJ, et al. Amino acid variation in HLA class II proteins is a major determinant of humoral response to common viruses. Am J Hum Genet. 2015 Nov 5;97 (5):738–743.

132. Voigt EA, Ovsyannikova IG, Haralambieva IH, et al. Genetically defined race, but not sex, is associated with higher humoral and cellular immune responses to measles vaccination. Vaccine. 2016 Aug 30;34(41):4913–4919.

133. Moss WJ. Measles. Lancet. 2017 Dec 2;390(10111):2490–2502.

134. Hull J, Campino S, Rowlands K, et al. Identification of common genetic variation that modulates alternative splicing. PLoS Genet. 2007 Jun;3(6):e99.

135. Zhao K, Lu ZX, Park JW, et al. GLiMMPS: robust statistical model for regulatory variation of alternative splicing using RNA-seq data. Genome Biol. 2013;14(7):R74.

136. Buchholz CJ, Gerlier D, Hu A, et al. Selective expression of a subset of measles virus receptor-competent CD46 isoforms in human brain. Virology. 1996;217(1):349–355.

137. Iwata K, Seya T, Ueda S, et al. Modulation of complement regulatory function and measles virus receptor function by the

serine-threonine-rich domains of membrane cofactor protein (CD46). Biochem J. 1994 Nov 15;304(Pt 1):169–175.

138. Buchholz CJ, Schneider U, Devaux P, et al. Cell entry by measles virus: long hybrid receptors uncouple binding from membrane fusion. Virology. 1996;70:3716–3723.

139. Ni Choileain S, Astier AL. CD46 processing: a means of expression. Immunobiol. 2012 Feb;217(2):169–175.

140. Ni Choileain S, Hay J, Thomas J, et al. TCR-stimulated changes in cell surface CD46 expression generate type 1 regulatory T cells. Sci Signal. 2017 Oct 24;10:502.

141. Flajnik MF, Kasahara M. Origin and evolution of the adaptive immune system: genetic events and selective pressures. Nat Rev Genet. 2010 Jan;11(1):47–59.

142. Oberg AL, McKinney BA, Schaid DJ, et al. Lessons learned in the analysis of high-dimensional data in vaccinomics. Vaccine. 2015; S0264–410X:574–575.

143. Hardy J, Singleton A. Genomewide association studies and human disease. N Engl J Med. 2009 Apr 23;360(17):1759–1768.

144. Wellcome Trust Case Control C. Genome-wide association study of 14,000 cases of seven common diseases and 3,000 shared controls. Nature. 2007;447(7145):661–678.

145. Yang J, Benyamin B, McEvoy BP, et al. Common SNPs explain a large proportion of the heritability for human height. Nat Genet. 2010 Jul;42(7):565–569.

146. Willer CJ, Schmidt EM, Sengupta S, et al. Discovery and refinement of loci associated with lipid levels. Nat Genet. 2013 Nov;45(11):1274–1283.

147. Nikpay M, Goel A, Won HH, et al. A comprehensive 1,000 Genomes-based genome-wide association meta-analysis of coronary artery disease. Nat Genet. 2015 Oct;47(10):1121–1130.

148. Al Olama AA, Kote-Jarai Z, Berndt SI, et al. A meta-analysis of 87,040 individuals identifies 23 new susceptibility loci for prostate cancer. Nat Genet. 2014 Oct;46(10):1103–1109.

149. Fuchsberger C, Flannick J, Teslovich TM, et al. The genetic architecture of type 2 diabetes. Nature. 2016 Aug 4;536(7614):41–47.

150. Biological insights from. 108 schizophrenia-associated genetic loci. Nature. 2014 Jul 24;511(7510):421–427.

151. MacArthur J, Bowler E, Cerezo M, et al. The new NHGRI-EBI Catalog of published genome-wide association studies (GWAS Catalog). Nucleic Acids Res. 2017 Jan 4;45(D1):D896–D901.
   • Describes the NHGRI-EBI Catalog of published genome-wide association studies.

152. McCarthy S, Das S, Kretzschmar W, et al. A reference panel of 64,976 haplotypes for genotype imputation. Nat Genet. 2016 Oct;48(10):1279–1283.

153. Pruim RJ, Welch RP, Sanna S, et al. LocusZoom: regional visualization of genome-wide association scan results. Bioinformatics. 2010 Sep 15;26(18):2336–2337.

154. Manolio TA. Genomewide association studies and assessment of the risk of disease. N Engl J Med. 2010 Jul 8;363(2):166–176.

155. Pe'er I, Yelensky R, Altshuler D, et al. Estimation of the multiple testing burden for genomewide association studies of nearly all common variants. Genet Epidemiol. 2008 May;32(4):381–385.

156. MacArthur DG, Manolio TA, Dimmock DP, et al. Guidelines for investigating causality of sequence variants in human disease. Nature. 2014 Apr 24;508(7497):469–476.

157. van de Bunt M, Cortes A, Brown MA, et al. Evaluating the performance of fine-mapping strategies at common variant GWAS loci. PLoS Genet. 2015;11(9):e1005535.

158. Ding K, Ij K. Methods for the selection of tagging SNPs: a comparison of tagging efficiency and performance. Eur J Hum Genet. 2007 Feb;15(2):228–236.

159. Stram DO. Tag SNP selection for association studies. Genet Epidemiol. 2004 Dec;27(4):365–374.

160. Marchini J, Howie B. Genotype imputation for genome-wide association studies. Nat Rev Genet. 2010 Jul;11(7):499–511.

161. Li Y, Willer C, Sanna S, et al. Genotype imputation [10.1146/annurev.genom.9.081307.164242 doi]. Am J Hum Genet. 2009;10:387–406.

162. Schaid DJ, Chen W, Larson NB. From genome-wide associations to candidate causal variants by statistical fine-mapping. Nat Rev Genet. 2018 Aug;19(8):491–504.

163. Sl S, Jc B. Strategies for fine-mapping complex traits. Hum Mol Genet. 2015 Oct 15;24(R1):R111–R119.

164. Rhee SY, Wood V, Dolinski K, et al. Use and misuse of the gene ontology annotations. Nat Rev Genet. 2008 Jul;9(7):509–515.

165. Harrow J, Frankish A, Gonzalez JM, et al. GENCODE: the reference human genome annotation for the ENCODE project. Genome Res. 2012 Sep;22(9):1760–1774.

166. The ENCODE. (Encyclopedia of DNA elements) Project. Science. 2004 Oct 22;306(5696):636–640.

167. Andersson R, Gebhard C, Miguel-Escalada I, et al. An atlas of active enhancers across human cell types and tissues. Nature. 2014 Mar 27;507(7493):455–461.

168. Kundaje A, Meuleman W, Ernst J, et al. Integrative analysis of 111 reference human epigenome. Nature. 2015 Feb 19;518 (7539):317–330.

169. Maurano MT, Humbert R, Rynes E, et al. Systematic localization of common disease-associated variation in regulatory DNA. Science. 2012 Sep 7;337(6099):1190–1195.

170. Millstein J, Zhang B, Zhu J, et al. Disentangling molecular relationships with a causal inference test. BMC Genet. 2009 May;27(10):23.

171. Zhu Z, Zhang F, Hu H, et al. Integration of summary data from GWAS and eQTL studies predicts complex trait gene targets. Nat Genet. 2016 May;48(5):481–487.

172. Giambartolomei C, Vukcevic D, Schadt EE, et al. Bayesian test for colocalisation between pairs of genetic association studies using summary statistics. PLoS Genet. 2014 May;10(5):e1004383.

173. Hormozdiari F, van de Bunt M, Segre AV, et al. Colocalization of GWAS and eQTL signals detects target genes. Am J Hum Genet. 2016 Dec 1;99(6):1245–1260.

174. Zilliox MJ, Moss WJ, Griffin DE. Gene expression changes in peripheral blood mononuclear cells during measles virus infection. Clin Vaccine Immunol. 2007;14(7):918–923.

175. Dhiman N, Ovsyannikova IG, Oberg AL, et al. Immune activation at effector and gene expression levels after measles vaccination in healthy individuals: a pilot study. Hum Immunol. 2005;66:1125–1136.

176. Haralambieva IH, Zimmermann MT, Ovsyannikova IG, 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(8):e0160970.
   • Assesses the gene expression changes in peripheral blood mononuclear cells during measles virus infection. Provides insights into important genes involved in the host response to measles.

177. Haralambieva IH, Kennedy RB, Simon WL, et al. Differential miRNA expression in B cells is associated with inter-individual differences in humoral immune response to measles vaccination. PLos ONE. 2018;13(1):e0191812.

178. Querec TD, Akondy RS, Lee EK, et al. Systems biology approach predicts immunogenicity of the yellow fever vaccine in humans. Nat Immunol. 2009;10(1):116–125.
   •• A landmark paper: one of the first reports applying a systems biology approach to predict response to vaccination (with yellow fever vaccine).

179. Nakaya HI, Wrammert J, Lee EK, et al. Systems biology of seasonal influenza vaccination in humans. Nat Immunol. 2011;12(8):786–795.

180. Kazmin D, Nakaya HI, Lee EK, et al. Systems analysis of protective immune responses to RTS,S malaria vaccination in humans. Proc Natl Acad Sci USA. 2017 Feb 28;114(9):2425–2430.

181. Papalexi E, Satija R. Single-cell RNA sequencing to explore immune cell heterogeneity. Nat Rev Immunol. 2018 Jan;18(1):35–45.

182. Friedensohn S, Khan TA, Reddy ST. Advanced methodologies in high-throughput sequencing of immune repertoires. Trends Biotechnol. 2017 Mar;35(3):203–214.

183. Hu Y, An Q, Sheu K, et al. Single cell multi-omics technology: methodology and application. Front Cell Dev Biol. 2018;6:28.

184. Stoeckius M, Hafemeister C, Stephenson W, et al. Simultaneous epitope and transcriptome measurement in single cells. Nat Methods. 2017 Sep;14(9):865–868.
185. Peterson VM, Zhang KX, Kumar N, et al. Multiplexed quantification of proteins and transcripts in single cells. Nat Biotechnol. 2017 Oct;35(10):936–939.
186. Hou Y, Guo H, Cao C, et al. Single-cell triple omics sequencing reveals genetic, epigenetic, and transcriptomic heterogeneity in hepatocellular carcinomas. Cell Res. 2016 Mar;26(3):304–319.
187. Hu Y, Huang K, An Q, et al. Simultaneous profiling of transcriptome and DNA methylome from a single cell. Genome Biol. 2016 5;May(17):88.
188. Frantz PN, Teeravechyan S, Tangy F. Measles-derived vaccines to prevent emerging viral diseases. Microbes Infect. 2018 Oct - Nov;20 (9–10):493–500.
189. Griffin DE. Current progress in pulmonary delivery of measles vaccine. Exp Rev Vaccines. 2014 Jun;13(6):751–759.
190. Griffin DE, Pan CH. Measles: old vaccines, new vaccines. Curr Topics Microbiol Immunol. 2009;330:191–212.
191. de Swart RL, de Vries RD, Rennick LJ, et al. Needle-free delivery of measles virus vaccine to the lower respiratory tract of non-human primates elicits optimal immunity and protection. NPJ Vaccines. 2017;2:22.
192. de Vries RD, Stittelaar KJ, Osterhaus AD, et al. Measles vaccination: new strategies and formulations. Expert Rev Vaccines. 2008;7 (8):1215–1223.
193. Joyce JC, Carroll TD, Collins ML, et al. A microneedle patch for measles and rubella vaccination is immunogenic and protective in infant rhesus macaques. J Infect Dis. 2018 Jun 5;218(1):124–132.
194. Edens C, Collins ML, Goodson JL, et al. A microneedle patch containing measles vaccine is immunogenic in non-human primates. Vaccine. 2015 Mar 12;S0264–410X(15):285–286.
195. Putz MM, Muller CP. The rationale of a peptide-conjugate vaccine against measles. Vaccine. 2003 Jan 30;21(7–8):663–666.
196. Gala RP, Popescu C, Knipp GT, et al. Physicochemical and preclinical evaluation of a novel buccal measles vaccine. AAPS Pharm Sci Tech. 2017 Feb;18(2):283–292.