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Expert Review of Vaccines

ISSN: (Print) (Online) Journal homepage: https://www.tandfonline.com/loi/ierv20

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**To cite this article:** Daniel O'Connor (2022) The omics strategy: the use of systems vaccinology to characterize immune responses to childhood immunization, Expert Review of Vaccines, 21:9, 1205-1214, DOI: 10.1080/14760584.2022.2093193

**To link to this article:** https://doi.org/10.1080/14760584.2022.2093193



Published online: 05 Jul 2022.

EXPERT REVIEW OF VACCINES
2022, VOL. 21, NO. 9, 1205–1214
https://doi.org/10.1080/14760584.2022.2093193

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REVIEW



# The omics strategy: the use of systems vaccinology to characterize immune responses to childhood immunization

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**ABSTRACT**

**Introduction:** Vaccines have had a transformative impact on child health. Despite this impact, the immunological processes involved in protective responses are not entirely understood and vaccine development has been largely empirical. Recent technological advances offer the opportunity to reveal the immunology underlying vaccine response at an unprecedented resolution. These data could revolutionize the way vaccines are developed and tested and further augment their role in securing the health of children around the world.

**Areas covered:** Systems level information and tools are now being deployed by vaccinologists at all stages of the vaccine development pathway; however, this review will specifically describe some of the key findings that have be gleaned from multi-omics datasets collected in the context of childhood immunization.

**Expert opinion:** Despite the success of vaccines, there remains hard-to-target pathogens, refractory to current vaccination strategies. Moreover, zoonotic diseases with pandemic potential are a threat to global health, as recently illustrated by COVID-19. Systems vaccinology holds a great deal of promise in revealing a greater understanding of vaccine responses and consequently modernizing vaccinology. However, there is a need for future studies – particularly in vulnerable populations that are targets for vaccination programmes – if this potential is to be fulfilled.

**ARTICLE HISTORY**
Received 30 April 2022
Accepted 20 June 2022

**KEYWORDS**
Systems vaccinology; genomics; transcriptomics; proteomics; microbiome; metabolomics

## 1. Introduction

Vaccines have had a profound impact on human health, as well as on societal and economic progress. The benefit of vaccines is particularly evident for child health, with the young being disproportionality affected by the morbidity and mortality associated with infectious diseases. Most vaccines in current use were developed and tested empirically, and there remains pathogens of global significance that, to date, have been refractory to vaccine development. Moreover, immune responses to vaccines given in early life can be weak, heterogeneous and wane rapidly. A better understanding of the mechanisms underlying protective immunity – particularly early in life – is key to developing new and improving existing vaccination strategies [1].

Technological advances present vaccinologists with unprecedented opportunities to dissect the immune responses to vaccines and transform the way vaccines are developed and tested by utilizing rational design. In recent years, there has been revolution in 'omics' technologies, enabling a comprehensive evaluation of the components of a given biological system, set or class, for example, the transcriptome is the study of all RNA molecules (mRNA, miRNA, etc). These so-called 'systems biology' approaches have been described as representing the dawn of a new era of hypothesis-enabling research as opposed to hypothesis-driven research [2]. A 'systems vaccinology' framework has been proposed as a strategy to leverage systems approaches in vaccine design and evaluation [3]. Leucocytes emigrant from tissue, including sites of vaccination, via the peripheral blood system on their way to lymphoid tissue [4]. Meaning there is an opportunity to sample these cells when they are circulating in blood, which is relatively easily to achieve by venepuncture. Consequently, peripheral blood is the principal material that has been interrogated in systems vaccinology studies, although a minority of studies have been conducted on lymphoid tissue (e.g. tonsillar material following elective tonsillectomy). In some studies, bulk tissue or whole blood is examined, while other studies split this material into its components or examined it at the single cell level. There are now several omic methods that assess the multiple layers of the immune system, this review will detail some of the key insights that have been gleaned from exploring these approaches in the context of childhood immunization.

### 1.1. Genome

The logical place to start with consideration of systems-based approaches into vaccine responses is with the human genome. Human genomes have been shaped by their co-evolution and interaction with microorganisms – both symbiotic and pathogenic. Consequently, our ability to fight-off

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**Article highlights**

- Interrogation of systems vaccinology data may elucidate novel correlates of vaccine-induced protection.
- Machine-learning and artificial intelligence can be utilised to predict vaccine responses.
- Integrative analyses of systems vaccinology data may reveal the immunological mechanisms underlying vaccines responses.
- Technological and analytical advances have the potential to revolutionise vaccine development and evaluation.

infection and respond robustly to vaccination is embedded in our genomes [5]. Indeed, studies of twins have shown vaccine responses to be highly heritable (i.e. have an important genetic component) [6,7]. Modern microarray and sequencing technologies have enabled the specific genetic determinants underlying these observational data to be elucidated. Recently, the first genome-wide association study (GWAS) of immunity to three childhood vaccines: capsular group C meningococcal (MenC), *Haemophilus influenzae* type b (Hib), and tetanus toxoid (TT) vaccines was published [8]. This study found genome wide significant associations between single nucleotide polymorphisms (SNP) proximal to the signal-regulatory proteins (SIRP) loci and the persistence of immunity following childhood MenC vaccine [8]. Interestingly, the lead SNP (rs6135736) associated with the phenotype was predicted be within an active enhancer region of two nearby genes – *SIPRG* and *SIRPB1*. Furthermore, this lead SNP was shown to be associated with the expression of both of these genes (expression quantitative trait loci; eQTL) [8]. *SIRPG* is the only SIRP family member expressed by T cells, and engagement of its protein product (SIRPγ) on T cells by CD47 on antigen-presenting cells enhances antigen-specific T-cell proliferation [9]. This study also found associations within the human leucocyte antigen (HLA) locus and TT-IgG concentrations; linking the classical HLA allele DRB1*0301 – which is distinct from the other major *HLA-DRB1* alleles both genetically and immunologically – to lower TT-IgG concentration following childhood immunization [8]. Intriguingly, DRB1*0301 is also predicted, using artificial neural networks to have the fewest strong and weak being affinity to TT epitopes [8].

A GWAS investigating the genetic determinants of adverse events following childhood immunization revealed SNPs within interferon-stimulated gene (*IFI44L*) and measles virus receptor (*CD46*) to be associated with MMR-related febrile seizure [10]. Interestingly, another GWAS reported SNPs within these same innate immune systems genes (i.e. *IFI44L* and *CD46*) were associated with measles-specific humoral immunity following MMR vaccine [11]. Intriguingly, the risk alleles (rs273259 and rs1318653) linked to MMR-related febrile seizures were also associated with higher measle-specific antibody titers post-vaccination [10,11]. GWAS studies of hepatitis B vaccine (HBV) responses have reproducibly linked SNPs within the HLA region and post-vaccination levels of anti-HBV surface antigen (HBsAg) antibody levels immune responses [12]. Interestingly, these genetic signals overlap with the associations observed in studies of chronicity of hepatitis B virus infection – suggesting individuals are predisposed to chronic infection by a genetically encoded inability to produce anti-HBsAg antibodies [13]. SNPs proximal to the *HLA-DPB1* gene have also been shown to be associated with infant HBV vaccine responses [14]. Determining the causal mechanism underlying these statistical associations remains challenging, but associated variants within the 3′ UTR of *HLA-DPB1* have been associated with the expression of this gene [15]. Moreover, specific amino acid variants within the HLA-DP protein that affect the electrostatic potential of an antigen-binding pocket have been linked to poor HBV vaccine responses – purportedly via differences in affinity to the HBsAg epitopes, and therefore presentation to CD4-positive T cells [16].

### 1.2. Epigenome

The epigenome is a term used to describe the heritable processes that regulate gene expression that are not encoded within the DNA sequence [17]. DNA methylation occurs at CpG (5′ cytosine-phosphate-guanine 3′) dinucleotides, and is involved in gene expression by inhibiting the binding of transcription factors or recruiting regulatory proteins [18]. Epigenetic modifications such as DNA methylation modulate both the development and function of the immune system [19]. Moreover, age-related differences in DNA hypomethylation is extremely apparent, and epigenetic differences may underly some of the age-related differences seen in adaptive and innate immune cells [20]. A study following a cohort of one-year-old infants through to the end of their second year described extensive longitudinal changes in the whole blood epigenome, and enrichment for pathways involved in regulation of cell–cell adhesion and T cell activation [21]. Moreover, this study found that hypermethylation of *IL-6* and hypomethylation of *HLA-DPB1* prior to pneumococcal vaccine was associated with higher anti-pneumococcal antibody concentrations [21]. Another study comparing whole blood DNA methylation of infants defined as 'normal' or 'low responders' to HBV vaccine found hypomethylation of CpG positions within *RNF39* – a transcription factor in the HLA class I region – was associated with poor HBV vaccine responses [22].

Non-coding RNAs are also considered a component of the epigenome. For example, microRNAs are small non-coding RNAs that regulate post-transcriptional expression of protein-coding genes [23,24]. Although miRNAs have been implicated in processes related to immune responses, there have been few studies investigating miRNAs in the context of human vaccine responses. One study examined miRNA in serum taken from children prior to and 21 days after either ASO3B adjuvanted split virion or a whole virion non-adjuvanted influenza vaccine [25]. This study used a microarray to quantify microRNAs, finding 19 miRNAs were differentially expressed post-vaccination after adjustment for multiple testing. However, many of these miRNAs have little evidence of existence in public databases and were not validated by real-time quantitative polymerase chain reaction (RT-qPCR) assay [25]. One miRNA, miR-142-3p was validated by RT-qPCR on but was subsequently not replicated in an additional cohort [25].

### 1.3. Transcriptome

Technological advances, initially in DNA microarrays, and then in next-generation sequencing (NGS) have facilitated an expansive examination of the transcriptome within biomedical research. Several studies have interrogated the blood transcriptome following childhood immunization – describing an early (~ 1 day post-vaccination) upregulation of genes associated with innate recognition of microbial motifs [26–28]. Moreover, a consistent plasma cell /immunoglobulin gene profile is apparent ~7 days after intramuscular vaccine administration [26–29]. Blood transcriptome profiling has also used to gain insight into the mechanisms underlying childhood vaccine reactogenicity and immunogenicity. A recent study of infants given the group B meningococcal vaccine (4CMenB) described an early gene signature linked to expression of genes involved in neutrophil recruitment (e.g. *SELL*) and post-vaccination fever [28]. This study also applied machine-learning approaches using baseline blood transcriptomic data to develop models able to predict the subsequent immunogenicity and reactogenicity of the 4CMenB vaccine [28].

Transcriptomic studies have been also utilized to contrast immune mechanisms underlying responses to differing vaccine platforms and formulations. Moderate protection against virologically confirmed influenza in children is observed following either trivalent inactivated influenza vaccine (TIV) or live-attenuated influenza vaccine (LAIV) [30]. However, stronger serum antibody responses, in terms of hemagglutinin inhibition assay titers, have been observed following trivalent inactivated influenza vaccine (TIV) compared with live-attenuated influenza vaccine (LAIV), which induces higher levels of local influenza virus-specific respiratory IgA – highlighting differences in the stimulation of antibody compartments between these vaccine platforms [31]. Transcriptomic analysis found that both these vaccines evoke the expression of IFN-signaling genes in peripheral blood but with differing kinetics, for TIV this is seen early at day 1 whereas this is seen later in the case of LAIV at day 7 [27,32]. Interestingly, for both vaccines, this IFN-related gene signal correlated with the fold-increase in antibody titers post-vaccination [26,27]. A further study comparing MF59-adjuvanted TIV (ATIV) to nonadjuvanted TIV in children, found more robust and homogeneous transcriptional responses to ATIV compared with TIV [26]. While this study generally found similar blood transcriptional modules (BTMs) one day after either of these vaccines, BTMs associated with monocytes, toll-like receptor and inflammatory signaling were higher following ATIV compared with TIV [26]. This study also suggested that ATIV immunization pushed childhood vaccine responses more toward the transcriptomic profile observed in adults following TIV [26]. Age has been linked to transcriptional responses, older children (9–18 years) having elevated expression of inflammatory pathway and inflammasome genes compared with younger children (3–8 years) following inactivated influenza vaccine [29]. This study also suggested that vaccination history impacted the innate response to vaccination, with children who received an influenza vaccine the previous year having somewhat decreased innate gene profiles [29]. HIV-infected children display lower ability to induce and maintain effective immunological responses to routine immunizations. Differences in the pre-vaccination gene expression profiles of $CD4^+$ T cell stimulated with influenza antigen have been observed between HIV-infected children who subsequently responded to seasonal influenza vaccine, compared with non-responders [33]. *IL21* expression was shown to be higher in peripheral T follicular helper cells from responders following in vitro stimulation with H1N1 compared with non-responders. In contrast, the expression of *CXCR4* by stimulated CD4 T cells was associated with non-responder status [33]. Moreover, despite quantitative similarities in B cell subsets in antiretroviral therapy (ART) treated individuals with stable viral control, sorted B cells from HIV-infected children differ at the gene expression level from age-matched healthy controls. Furthermore, transcriptomic signatures in baseline resting memory B cells predict subsequent responses to influenza vaccination [34,35]. Children who respond to influenza vaccine have higher pre-vaccination expression of gene sets associated with regulation of adaptive immune responses and B cell receptor signaling, compared with non-responders [34]. These data highlight despite ART treatment and overall normality in B cell frequencies, suboptimal B cell function persists that is illuminated by gene expression profiling.

The live-attenuated rotavirus (RV) vaccine is another childhood vaccine – like LAIV – where the immunological mechanisms underlying protection are unclear, although serum RV-specific IgA titers have been associated with protection [36]. A recent study evaluated host transcriptome responses to RV vaccine (Rotateq) and compared these with children infected with rotavirus and healthy controls [37]. This study showed similar molecular responses induced by vaccine and wild-type infection, including over-expression of genes associated with gastrointestinal disease and inflammation. However, machine-learning approaches using blood transcriptome date were able to accurately distinguish vaccination and natural infection [37].

### 1.4. Immunome

Adaptive immunity is facilitated by antigen receptors on B and T cells, which are generated through a process of somatic recombination. This results in extremely high levels of diversity and the potential to recognize a broad array of antigens. Recent advances in NGS technologies have enabled the in-depth characterization of immune receptor repertoires (immunome) [38,39]. A study of antibody repertoire before and after following influenza vaccination, across several age groups, found TIV or LAIV is associated with a relative decrease in IgM usage and a relative increase in IgG and IgA – dues to changes in antibody transcript copy number [40]. Individuals who received LAIV had less of a change in relative IgM usage compare with TIV recipients. Moreover, children who received TIV were more likely to increase relative IgA usage compared

with adults [40]. A longitudinal study examined changes in the BCR repertoire in early-life and how these are impacted by environment exposures [41]. This study observed a time-dependent linear accumulation of somatic hypermutation (SHM) of the antibody genes over the first 3 years of life, and whereas IgM and IgD isotypes reached adult levels of SHM by 2 years of age, IgA and IgG progressively gained mutations and were <75% as mutated as adults by 3 years of age [41]. This study identified B cells specific for vaccine antigens, showing tetanus toxoid (TT)-specific clones, including unswitched IgM expressing clones with a wide-range of SHM frequencies – suggesting an a role for IgM in early childhood response to this vaccine antigen [41]. This study also showed increased IgG1 subclass and decreased IgG2 subclass in early life compared with adults, showing that IgG2 + B cells did not increase by three years of age and suggested this may underly impaired responses against polysaccharide antigens in young children [41,42].

Few studies have employed NGS technologies to characterize the T cell receptor (TCR) repertoire in childhood or described how this may change in early life – in response to vaccination or infection. In early life the majority of T cell are naïve T cells that newly emerge from the thymus [43]. Repeated antigenic exposures during childhood, along with thymic involution, result in age-related changes in the relative abundance of memory T cells compared with naïve T cells [43]. The public TCR repertoire is assumed to largely represent convergence due to common antigenic challenges; albeit some public clones have been described as fetal clonotypes that persist into adulthood [44]. Indeed high-throughput screening of public TCR ß sequences has been able to identify sequences associated with cytomegalovirus infection in a cohort of children and adults [45]. Cross-sectional TCR ß repertoire profiling of individuals varying from childhood to old age found near linear decreases in TCR diversity [46]. Mathematical modeling has suggested antigen exposures early in life have particularly profound effects on shaping the immune repertoire, with perinatal clonal expansion leaving a long-lasting imprint on the T cell repertoire [47]. Moreover, studies have shown the importance of childhood hemagglutinin imprinting, via the first influenza infection children are exposed to, on the susceptibility to severe disease following infection with novel hemagglutinin (HA) subtypes in the same phylogenetic group [48]. However, to date, there's a paucity of data on the T cell repertoire following childhood vaccination and how this relates to the robustness and successfulness of vaccine responses.

### 1.5. Proteome

The proteome constitutes the functional translation of the information contained within the genome. Proteins have various direct effector functions both in their soluble and cell associated forms. This feature makes them particularly appealing in the pursuit of the biochemical mechanisms underlying health and disease, as well as potential therapeutic targets. The most obvious proteins to be considered within the context of immune responses are the immunoglobulins – the secreted form of the BCR – these have a broad repertoire of specificities that reflects an individual's antigenic exposure. High-throughput methods of characterizing this antigenic experience have been developed; for example, using immunoprecipitation and massively parallel DNA sequencing of a bacteriophage library displaying microbial peptides [49]. A recent study, using a proteome-wide peptides from all known human viruses, found unvaccinated children who acquired measles lost up to 73% of their antibody repertoire – this depletion was not observed after MMR (measles, mumps, and rubella) vaccination [50]. This study provided a link in the mechanistic chain – reduction in humoral immune memory after measles infection – to support previous epidemiological data that associated measles infection with increased morbidity and mortality for years after infection [51]. A microarray of the measles virus proteome has also been used to profile IgG measle-specific humoral immune responses following childhood MMR vaccination [52]. This study associated antibody responses to measles P, N, L-s3 and F proteins with measles virus neutralization titers [52].

Antibody binding is determined by the fragment antibody binding (Fab) region, but these proteins contain another region called the fragment crystallizable (Fc) that dictates their functional capacity – the study of antibody functionalities has recently been coined 'systems serology' [53]. Maternally derived antibodies provide some protection against infectious disease early in life; maternal immunization results in the transfer of vaccine-specific antibody to the child and can offer protection against neonatal tetanus and severe pertussis [54]. However, not all antibodies are transferred across the placenta equally and Fc features have been associated with determining their transfer [55]. These data highlight the potential for vaccines to be designed to maximize the placental transfer of protective antibodies to offspring. Furthermore, protection may require particular antibody functionalities; for example, bactericidal antibody is associated with protection against meningococcal disease, and opsonophagocytic antibodies may be required to protect against pneumococcal disease [56]. The mechanistic vaccine-induced correlates of protection (CoP) can be difficult to determine; consequently, some remain unknown and others controversial. The use of systems serology holds some potential in clarifying some of the elusive and uncertain CoP, which would aid in both development of new as well as the evaluation of current vaccination strategies. A recent study of Fc-effector functions following childhood measles vaccination found differences in the persistence of Fc-effector functions depending on when MMR vaccine was administered [57]. Children who received an early dose of MMR at 6–8 months of age had a more rapid decay in antibody polyfunctionality compared with children who received MMR at 14 months of age [57]. Influenza virus-specific antibody-dependent cellular cytotoxicity (ADCC) has been detected in some individuals against strains for which they lack neutralizing activity, leading to suggestions that influenza-specific ADCC antibodies may provide cross-protection against multiple influenza strains [58]. ADCC activity is not expected to prevent cells from being infected but may aid in the elimination of infected cells; consequently, accelerating pathogen clearance and reducing disease

severity. Modest increases in ADCC activity have also been shown following childhood immunization with both TIV and LAIV in children, but there are conflicting data, and this increase seems contingent on prior influenza immunizations – so it remains unclear how important this effector function is in childhood influenza vaccine-induced protection [59,60]. Changes in the level of Fc *N*-glycosylation of antigen-specific IgG1 have been described following childhood influenza (TIV) and tetanus immunization, characterized by increased galactosylation and sialylation and decreased bisecting N-acetylglucosamine (GlcNAc) [61]. Interestingly, the decrease in bisecting GlcNAc might suggest lower ADCC potency for vaccine-specific IgG1 [61].

Beyond antibodies, the plasma proteome contains a complex milieu of components with various immunological properties. Plasma is readily and routinely collected for clinical and research purposes, and contains the most complex human-derived proteome – containing proteins that function in circulation as well as proteins that are either being transported through, or have leaked into, the circulation [62]. The advent of high-throughput tandem mass spectrometry, protein microarrays and multiplexed immunoassays has facilitated detailed proteomic analysis of plasma and serum (the protein solution remaining after blood is allowed to clot). The plasma proteome has been used to describe the trajectory of the newborn immune system – describing a general increase in classical complement components, decrease in lectin and alternative complement pathway components within the first week of life [63]. Cord blood collected following preterm delivery has been shown to have a distinctly proinflammatory signature characterized by high levels of CXCL11 and IL-8 compare with term deliveries [64]. This study also described widespread changes in the plasma proteome from birth to three months of age, but that the plasma proteome of preterm and term infants had largely converged by the latter timepoint [64].

A recent study found infants who received the 4CMenB vaccine concomitantly with other routine infant immunizations (PCV13 and DTaP-IPV-Hib) had increased plasma levels of acute phase reactants, as well proinflammatory cytokines and proteins that promote mobilization of neutrophils, compared with infants who received routine infant immunizations alone [28]. Infant 4CMenB vaccination is commonly associated with fever, while this this is generally mild and benign this can result in parental and even healthcare professional concern. The aforementioned study found elevated levels of CRP and neutrophil counts – commonly used as diagnostic markers in suspected sepsis – 24 hours post-vaccination with 4CMenB, which could have implication in the assessment of febrile infants following vaccination [28].

### 1.6. Microbiome

The human body shares its ecosystem with a complex multispecies microbial community – collectively referred to as the holobiont or superorganism [65]. In recent years, the microbial contribution to both health and disease has become increasingly apparent. The microbial exposure during the postnatal and early infant period has been shown to have a critical role in the maturation and education of the host immune system, with ongoing effects on susceptibility to immune-related disease later in life [66]. Given the importance of the microbiome to the immune system, it has been hypothesized that this may also impact vaccine responses. Moreover, it has been speculated that differences in the intestinal microbiome could partly underly geographical differences seen in the vaccine efficacy – particularly oral vaccines such as rotavirus vaccines (RVV) and the oral polio vaccine (OPV). Indeed, differences in the composition of the gut bacterial phyla have been observed between responders and non-responders to both RVV and OPV [67,68]. High abundance of the Bacilli phylum and low abundance of *Bacteroides* have been correlated with RVV responsiveness in Ghanaian infants [67]. A pilot study in a small number Pakistani infants found a relatively high abundance of Proteobacteria was associated with RVV responses, and tentatively suggested this may complement the findings from Ghana, speculating LPS derived from Proteobacteria may have greater immune-stimulatory properties than *Bacteroides* [69]. However, a recent study in Zimbabwean infants did not find an association between the composition of the gut microbiome to RVV immunogenicity, although they did describe a species level association between *Bacteroides thetaiotaomicron* and anti-rotavirus IgA titers [70]. Likewise, in Indian infants no consistent differences were seen in the microbiome composition between RVV responders and non-responders [71]. An association between higher abundance of *Actinobacteria* and OPV responses has been reproduced in Chinese and Bangladeshi infants [68,72]. However, in a double-blinded randomized placebo-control trial, no difference was seen in seroconversion rates in Indian infants who received the antibiotic azithromycin prior to OPV, despite the changes this has on the infant microbiome [73]. Considerable heterogeneity has been observed in the findings of the studies to date examining the relationship between the host microbiome and vaccines responses – likely largely reflecting the heterogeneity of the study designs, methods, and populations – but it is clear future studies are required to further elucidate the impact of the microbiota on vaccine responses.

Vaccines can have a reciprocal impact on the microbiome – both specifically on the vaccine target as well as conceivably due to other organisms replacing this ecological niche. Pneumococcal immunization is followed by a decrease in vaccine serotype pneumococci in the nasopharynx of healthy infants but has also been associated with an increase in streptococci and anaerobic bacteria [74]. A further study of infants with acute respiratory infections, suggested children who had previously received pneumococcal vaccination had an increase in *Streptococcus* genera in the nasopharynx [75]. However, this association was not statistically significant, and another study reported pneumococcal vaccination did not significantly alter the nasopharyngeal microbiome and suggested limited replacement with pathogens other than non-vaccine type pneumococci [75,76].

### 1.7. Metabolome

Intracellular metabolic pathways play a key role in immune cell function and studies profiling the metabolic state in early life and childhood are incipient [77,78]. The neonatal period represents both a transition from a low microbe to a relatively high microbe exposure environment, as well as a state of high energy demand with little energy reserve. Consequently, it has been hypothesized that the neonatal immune system is skewed toward a tolerogenic state via metabolic adaptations, to limit the high demands of immune responses [79]. Indeed, dramatic changes in the plasma metabolome have been observed in the first week of life, such as purine metabolism [78]. Neonates have elevated levels of adenosine due to a relative deficiency in adenosine deaminase 1, which has been linked to the anti-inflammatory and Th2 skewing bias observed in newborn infants, compared with adults [80,81]. Moreover, cord blood macrophages have been shown to have altered metabolic pathways with defective anaerobic glucose metabolism and reduced mTOR activation, a state that can also be induced in adult macrophages by incubation with S100A8/A9, which is highly abundant in neonatal serum [82]. Cord blood monocytes stimulated with Bacillus Calmette–Guérin (BCG) vaccine have also been shown to have tolerogenic responses following stimulation with LPS, compared with adult monocytes [83]. These BCG-trained neonatal monocytes demonstrate impaired lactate production, which is a metabolite that has been linked to 'train immunity' in adults [83]. Further supporting the importance of the study of immunometabolism are the various inborn errors of metabolism associated with immunodeficiencies – with features including poor vaccine responses – that can be resolved when the metabolic defect is corrected [84,85]. The application of metabolomics approaches to vaccine responses is still in its infancy but have considerable potential, particularly when integrated with other layers of omics data.

### 1.8. Cytometry

High-dimensional single-cell immune profiling, initially using flow cytometry and more recently mass cytometry has been a mainstay tool for the evaluating immune responses [86]. Immunological mechanisms of vaccine-induced protection remain poorly understood for some vaccines such as the influenza and typhoid vaccines [56,87]. High-dimensional single-cell analyses have been used to explore the many immune cells involved in both humoral and cell-mediated immunity (CMI) induced following vaccination. Age-associated differences have been seen in CMI following live-attenuated typhoid vaccine (Ty21a) [88]. Children and adults both displayed increases in percentage $CD69^+$ expression by CD4 T effector memory ($T_{EM}$) following Ty21a, but this study showed only adults have a statistically significant increase in $CD69^+$ expression by CD8 $T_{EM}$ [88]. Nonetheless, children and adults did appear to have similar magnitude of *S.* Typhi-responsive circulating $CD8^+$ multifunctional T cells, which have previously been associated with protection against typhoid fever [89].

LAIV induces early (3–7 days) activation of tonsillar follicles and influenza-specific T follicular helper-($T_{FH}$)-cell responses in children [90]. Compared with adults, children's tonsillar $CD4^+$ T cells had a higher percentage of bona fide $T_{FH}$ cells [90]. Moreover, a positive correlation was observed between LAIV induced tonsil $T_{FH}$-cell responses and systemic influenza-specific IgG induction [90]. Interestingly, a study that collected tonsils from children ~9 days after inactivated influenza (split virion) also described an increased frequency of $T_{FH}$-cells and that these correlated with the frequency of influenza antigen-specific IgG antibody-secreting cells [91]. $CXCR5^+$ $CD4^+$ T cells share some of the functional properties of $T_{FH}$ cells and are found circulating in the peripheral blood [92]. Following the childhood immunization with inactivated influenza vaccine there is an increase in the frequency of peripheral blood $CD4^+$ T cells expressing inducible T cell costimulator (ICOS) – an important molecule for $T_{FH}$ development – peaking at seven days [92]. Moreover, the increase in $ICOS^+CXCR3^+CXCR5^+CD4^+$ T cells – distinct from bona fide $T_{FH}$ cells by their lack of BCL-6 – were correlated with the increase of hemagglutination inhibition (HI) titers post-vaccination [92]. However, this study suggested that this association is restricted to recall responses (i.e. previously encountered influenza strains) and not primary antibody responses, as no correlation was not seen to H1N1 virus, which the study children would not have been exposed to prior to vaccination [92].

## 2. Conclusion

Vaccination is a pillar of public health policy, particularly in relation to child health. Much of the success of vaccines has been built empirically, without a clear understanding of the immunological mechanisms underlying vaccine efficacy. This limited understanding has hampered vaccine development, and contributed to the failure to develop vaccines against some recalcitrant pathogens (e.g. human immunodeficiency virus) [1]. However, novel vaccine platform technologies (e.g. mRNA and viral vector vaccines) have recently been deployed during the COVID-19 pandemic and have been shown to be highly efficacious [93,94]. These new vaccine technologies may indeed hold some promise in improving current vaccines, as well as in the development of vaccines for pathogens that have, to date, been elusive. Moreover, they provide an elegant example of the substantial impact new technologies can have on the field of vaccinology.

Recent technological innovations have enabled the dissection of immunological responses at an unprecedented resolution. Concurrently, there have been advances in computational approaches to analyze these large and complex datasets. Machine learning (ML) algorithms and artificial intelligence (AI) has increasingly been exploited to model and predict vaccine responses from systems datasets. Furthermore, software packages with user-friendly graphical user interfaces, such as SIMON (Sequential Iterative Modeling Over Night), have been developed to help immunologists explore their dataset using a suite of ML methods [95]. Consequently, systems level information and the tools needed to explore these

data are increasingly accessible to vaccinologists, at all stages of the vaccine development pathway. For example, in the early stages of preclinical vaccine development system vaccinology techniques may enable streamlining of vaccine candidates for progression to the next stages of vaccine development. Equally, in early phase clinical trials, these methods could enable a detailed examination of vaccine responses in a small number of individuals, which may inform decisions and de-risk progression of candidates to the latter stages of clinical trials – that are hugely expensive.

## 3. Expert opinion

Technological advances continue to present new and improved approaches to characterize the immune responses underlying vaccine responses. There has been a recent explosion in the use of single cell sequencing methods within the field of immunological research. While the high costs associated with single cell RNA-sequencing (scRNA-seq) have prohibited its widespread adoption, decreasing prices and an increasingly appreciation of the granularity these approaches provide, appear to indicate that scRNA-seq will likely soon dominate transcriptomic research. Moreover, cellular indexing of transcriptomes and epitopes by sequencing (CITE-seq) allows detection and quantification of multiplexed protein markers simultaneously with transcriptomic data, enriching the data that can be gleaned from these single cell experiments [96].

Future studies that collect multiple orthogonal omic datasets following vaccination will enable a multilayer integrative network description of immune responses. This will require advanced computational approaches to integrate rather than simply aggregate information from these various sources. To date, few studies have attempted to truly integrate multi-omics data collected from vaccine studies. However, tools such as DIABLO (Data Integration Analysis for Biomarker discovery using Latent cOmponents) and MMRN (Multiscale, Multifactorial Response Networks) have started to be applied to systems vaccinology datasets [97,98]. Furthermore, powerful methods such as tensor decomposition have recently been shown to be highly informative in understanding immune responses during COVID-19, when applied to rich multi-omic dataset [99].

Hitherto, the majority of systems vaccinology data have been collected from healthy adults in high income countries; yet, the targets of most vaccines in current use are at the extremes of age and the main burden of infectious disease morbidity and mortality lies within low- and middle-income countries [100]. Moreover, there are a paucity of studies applying these tools in vulnerable populations such as pregnant women, preterm infants, and individuals with chronic disease [101]. This does bring into to question the generalizability of current data to different age, geographical, genetic and health backgrounds. For example, nutritional status has an impact on the immune system, with undernutrition – which affects >800 million people globally – being associated with increased susceptibility to infections as well as poorer vaccine responses [102]. Breastfeeding has a considerable impact on immune programming in newborn infants [103]. Moreover, immune stimuli such as infection or vaccination – particularly early in life – may have long-term, nonspecific consequences on the immune system [104]. Future studies are needed to apply the promising systems approaches to understand immune response in more diverse population, enriching for individuals within populations that are targets for vaccination programmes.

This review described a systems vaccinology framework for both understanding and predicting vaccine immunogenicity and reactogenicity, which could have important implications in future vaccination strategies. It seems tractable that the immunological insights from these types of studies will be used in the rational design of future vaccine antigens and adjuvants. These approaches could be utilized at all stages of the vaccine developmental pathway, streamlining of development of the vaccine candidates with the best characteristics by maximizing the information available at important stages of decision-making.

## Author contributions

D O'Connor is solely responsible for the conception and design of the review article and interpreting the relevant literature.

## Declaration of interests

D O'Connor receives funding from the National Institute for Health and Care Research (NIHR) Oxford Biomedical Research Centre (BRC). The views expressed are those of the author and not necessarily those of the NHS, the NIHR or the Department of Health. The author has 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.

## Funding

This paper was not funded.

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