REVIEW PAPER

SYSTEM VACCINOLOGY: APPLICATIONS, TRENDS, AND PERSPECTIVES

By

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ABSTRACT

Systems vaccinology is an emerging field that integrates structural biology and immunology to revolutionize vaccine development and evaluation. By leveraging high-throughput omics technologies, computational biology, and bioinformatics, systems vaccinology offers comprehensive insights into the molecular and cellular mechanisms underlying immune responses. This holistic approach enables the identification of novel biomarkers, the prediction of vaccine efficacy, and an understanding of adverse reactions. Recent applications in infectious diseases, cancer immunotherapy, and personalized vaccines have demonstrated the potential of systems vaccinology to enhance vaccine design and optimize immunization strategies. Trends highlight the integration of multi-omics data, machine learning algorithms, and big data analytics to create predictive models of vaccine responses. The application of systems vaccinology has led to the discovery of correlates of protection, aiding in the rational design of next-generation vaccines. Despite these advancements, challenges remain, including data integration, standardization, and the need for interdisciplinary collaboration. Future perspectives focus on the development of universally accepted vaccines, rapid-response systems for emerging pathogens, and the incorporation of artificial intelligence to accelerate vaccine discovery. Systems vaccinology stands at the forefront of transforming public health by providing robust tools to combat infectious diseases and enhance global immunization programs.

Keywords: Systems Vaccinology, Omics Technologies, Vaccine Development, Immune Responses, Biomarkers, Predictive Models, Personalized Vaccines, Data Integration, Machine Studying, Big Data Analytics.

INTRODUCTION

Systems Vaccinology (SV) is an emerging science, evolving from systems biology, that aims to understand the effects of vaccines on the entire host system. Unlike systems biology, SV is highly application-focused and differs primarily from the more commonly used term "systems immunology." Like other areas of systems biology, SV seeks to overcome reductionist thinking and generate a more comprehensive, dynamic, and, in a sense, practical understanding of how different components of an organism interact, specifically in the context of vaccine-induced responses or factors influencing vaccine efficacy.

Viewing the host as a system interacting with its environment (consisting of multiple interacting systems) enables the identification of critical signatures necessary

for defining or achieving therapeutic success. Since it is embedded in a complex system, understanding host factors and their interaction with the environment in a holistic view is crucial for rational drug design, including structure-based vaccines.

This is particularly important in the case of chronic infections, where the pathogen establishes a continuous yet detrimental relationship with the host system. Understanding the host-pathogen interface is another vital area within systems vaccinology, potentially bridging the gap to other areas of pharmacology and further exposing the dynamics of this interaction. Chronic infections are typically established by pathogens using mechanisms that render host defenses partially ineffective, either by altering host immunity or by employing immunological stealth. Similarly, most cancer vaccines (with a few exceptions) have not yet proven highly successful. Applying SV to understand the complex interactions between cancer and its environment, such as the tumor stroma, can facilitate the development of more effective cancer vaccines.

While vaccines have been widely successful, the modes of action for many commonly used vaccines are still not well understood. Infectious diseases like HIV, malaria, tuberculosis, and dengue fever have shown resistance, at least partially, to traditional vaccination approaches. Systems vaccinology provides powerful tools to monitor a vast array of data types through omics technologies, reconstructing a comprehensive view of the processes leading to either the success or failure of a vaccine or vaccine prototype.

SV offers deeper insights into the processes leading to or preventing protective immunity, establishing a foundation for vaccine developers to overcome old challenges (Pulendran, 2014; Rappuoli & Aderem, 2011). This understanding becomes even more complex as sources of variation in immune responses, such as genetic diversity, environmental and psychological factors, the influence of the microbiome, and socioeconomic conditions (including obesity, diabetes, and malnutrition), become more evident and tangible.

Systems vaccinology enables a deeper understanding of the ongoing pathomechanisms by comparing successful immune responses, which can lead to the development of new immunization strategies for diseases without commercially available vaccines. Successful vaccines that trigger the immune system can be modeled based on expression signatures or molecular mechanisms that clear pathogens during infection. Data from entire populations, including non-responders, should inform vaccine design. The type and intensity of immune reactions can, among other factors, be efficiently modulated by Pattern Recognition Receptors (PRRs), such as Toll-like receptors (TLRs) and nucleotide-binding oligomerization domain (NOD) proteins, which are among the best-known adjuvant targets.

Understanding immune-evasive mechanisms and the latest immunological insights, along with the mechanisms of novel adjuvants, will lead to reprogramming ineffective responses into effective immunity. The applications of systems vaccinology include:

Immunogenicity and Protection: Predicting immunogenicity and optimal protection of vaccines in individuals based on host samples taken before or after vaccination. Applications include patient stratification for clinical trials, early identification of non-responders (allowing for alternative treatments), and risk assessment of adverse effects.

Vaccine Development: Accelerating vaccine development by taking comprehensive immunological snapshots during research, reducing the risk of failure in later clinical trials, and improving our understanding of immunity in vulnerable groups, such as young children and the elderly. This also includes identifying novel formulations and adjuvants, optimizing dosage (both relatively and absolutely), and enhancing our understanding of the relationship between immunity, metabolism, and the nervous system.

Complex Interactions: Exploring the interface between vaccines and complex phenomena like cancer and autoimmunity, including autoantibody levels and antibody subclasses. Understanding vaccines' potential

to modulate diseases with inflammatory components, such as neoplastic disorders, stroke, and heart disease, could have drastic public health benefits. This extends to the concept of using Self-Associated Molecular Patterns (SAMPs) to reduce inflammation, which seems feasible thanks to recent insights into the glycobiology of antibodies, including the anti-inflammatory effects of targeted Fc receptors.

1. The Study

- The integration of omics technologies, including genomics, proteomics, and metabolomics.
- The role of computational models in predicting vaccine responses.
- Challenges in the field, such as standardization and data integration.

2. Information and Prediction of Vaccine Responses

The maximum utility of systems vaccinology to date has been the identification of critical parameters related to immunogenicity and the most effective protection offered by a vaccine. Various studies have aimed to define system signatures, specifically based on gene expression analysis, that can serve as surrogates for vaccine efficacy. The application of these signatures could allow for:

- Evaluation and optimization of vaccine candidates, including the selection of adjuvants or dosage, primarily based on the observation of signatures in PBMCs in vitro.
- Early identification of non-responders after vaccination.

Meanwhile, the concept has been expanded to include characteristics of the host prior to vaccination. Some molecular and other properties have been identified to enable the prediction of vaccine response before treating a subject. This has thus far been aimed at two related practical applications:

- Stratification of patients for clinical trials based on their likelihood of responding to a vaccine.
- Early identification of potential non-responders before vaccination, allowing for the selection of alternative vaccine products, dosages, or administration regimens. Both of these options represent a form of personalized treatment.

A third related application could be the early identification of candidates for adverse side effects, including rare conditions such as narcolepsy. The highly effective yellow fever vaccine, YF-17D, was the first subject in a study of systems vaccinology where vaccinology and immunology were combined (Pulendran, 2009; Pulendran & Ahmed, 2011; Pulendran et al., 2013; Querec et al., 2009). Querec et al. (2009) succeeded in defining and verifying early post-vaccination gene expression signatures that predicted YF-17D adaptive immune responses, particularly CD8+ (cytotoxic) T-cell responses with up to 90% accuracy and neutralizing antibody responses with up to 100% accuracy, depending on the analysis technique. These predictions required models involving only a few parameters each.

Given the widespread use of this vaccine, it may also be a good candidate for further understanding and prediction of adverse side effects, including rare serious adverse effects (Barrett & Teuwen, 2009; Biscayart et al., 2014; Monath et al., 2005). Technologically, this initial study has shaped the conceptual approach to systems vaccinology. The authors combined FACS characterization of immune cells from patients sampled after vaccination in a time series with a multiplex panel of cytokines and gene expression analysis of PBMCs using microarrays. Gene expression data were analyzed for statistically significant changes using analysis of variance (ANOVA) across the entire time series of fold changes. Results were corrected for multiple testing, yielding a set of 65 genes putatively differentially regulated in PBMCs after vaccination. RT-PCR was used to validate the differential expression of a subset of these genes.

For the biological interpretation of microarray analysis results, initially fluctuating regulated genes were subjected to gene enrichment analysis using DAVID to identify enriched pathways and modules (Huang et al., 2007). Furthermore, transcription factor-binding sites statistically over represented in the promoters of these

genes were identified using TOUCAN (Aerts et al., 2003). Acquired immune reactions were characterized by epitope-specific T-cell assays and the determination of neutralizing antibody titers. However, the 65 differentially regulated genes were not sufficient to predict the magnitude of CD8+ T-cell responses. For this reason, expression fold changes of individual genes on day 3 and separately on day 7 were individually tested for significant correlation with later CD8+ T-cell responses and neutralizing antibody responses.

In addition to PCA analysis, which indicated a segregation of high and low CD8+ responders, average linkage hierarchical clustering analysis was used and confirmed the segregation of these two groups, followed by feature selection, model building, cross-validation, and further independent validation. Based on samples before and after vaccination, Nakaya et al. (2011) demonstrated that the signatures of protection between Live Attenuated Influenza Vaccines (LAIV) and Inactivated Influenza Vaccines (TIV) differ, with the authors specifically highlighting IFN-related genes as differentiating signatures following LAIV administration (Nakaya et al., 2011). Similar to Querec et al. (2009), this study aimed to predict vaccine efficacy based on samples taken a few days after vaccination, allowing the prediction of hemagglutinin-inhibiting (HAI) titers a month later, and specifically to enable the identification of vaccine nonresponders as early as possible.

Time, this study also focused on gene expression analysis (microarrays and RT-PCR) and FACS. The researchers individually mined gene expression data for PBMC subsets separated by FACS. This approach is particularly reflective of the functionally differentiated nature of PBMCs and may lead to the identification of marker signatures that would otherwise remain undetectable in bulk analyses due to expression primarily in critical but low-abundance subpopulations, as demonstrated here by plasmacytoid dendritic cells (pDCs). Additional cost-effective and commonly used analysis methods capable of differentiating cell types were proposed, notably a meta-analysis procedure by Nakaya et al. (2011) and the statistical deconvolution method using Cell type-Specific Significance Analysis of Microarrays (CSSAM) (Shen-Orr et al., 2010).

In Nakaya et al. (2011), a meta-analysis suggested a TIV-specific pattern of highly expressed genes in Antibody Secreting Cells (ASCs), whereas cell types implicated in the LAIV analysis were specific T-cells and monocytes. These results underscore the importance of different cell types in the analysis and how they modulate pro-protective mechanisms during vaccination. A similar study of TIV, without differentiating cell types, was performed by Bucasas et al. (2011), which also indicated a distinct gene expression signature comprising 494 genes segregating high and low vaccine responders (Bucasas et al., 2011). Likewise, in a study conducted by Vahey et al. (2010) for predicting the protectivity of a malaria vaccine, the immunoproteasome and apoptosis were identified as key processes, depending on the time post-vaccination (Vahey et al., 2010).

Furman et al. (2014) worked to define markers of immunological health, with the idea of using the intensity of immune reactions to an influenza vaccine as a measure of health (Furman et al., 2014; Tsang et al., 2014). Notably, they identified nine features available before vaccination that predicted vaccine response with 84% accuracy, along with several other parameters that were individually predictive to some extent but did not further improve the model. The data analyzed included the patient's age, whole-blood gene expression profiles, peptide-specific anti-influenza antibody titers measured via peptide microarrays, 50 cytokines and chemokines, and typing to a resolution of 15 immune cell subtypes using FACS. The authors found that age, taken alone, was the most informative predictor of HAI titers, with increased age correlating to reduced titers. As previously reported by others, pre-existing HAI titers (e.g., from prior vaccination or natural infection) were negatively associated with influenza vaccine-induced HAI titers, reportedly due to limited Dendritic Cell (DC) antigen presentation caused by pre-existing memory T-cells through natural killer (NK) cells. Furman et al. were able to specify the number of peptides predictive of this effect before vaccination and those that provided

independent information to predict vaccine efficacy.

Additionally, whole-blood gene expression analysis followed by gene module analysis suggested several gene sets where expression was negatively correlated with post-vaccination HAI titers, while one set associated with apoptosis was positively correlated. The identification of apoptosis as an important parameter echoes the results in malaria. Relevant data for predicting vaccine response were also present in levels of soluble Fas ligand (sFasL) and IL-12p40, and in the frequency of central memory CD4+ and effector memory CD8+ T-cells. From multiple perspectives, this study can be seen as exemplary in identifying predictive models of vaccine efficacy, particularly due to the breadth of omics techniques applied. Furman et al. (2014) measured (epitope-specific) antibody titers based on peptide arrays, gene expression analysis of blood cells, and FACS-based immune cell subtyping.

Tsang et al. (2014) compared pre- and post-vaccination PBMCs using FACS and showed that pre-vaccination cell population frequencies alone could predict antibody responses to TIV (Tsang et al., 2014). FACS-based discrimination of immune cell types followed by microarray analysis and cytokine panels led to the identification of PD-1+ CXCR5+ CD4+ T-cell numbers as indicators of the emergence of broadly HIV-neutralizing antibodies. This population is similar to CD4+ T follicular helper (Tfh) cells, which are critical for B-cell maturation in Germinal Centers (GC), indicating the potential to characterize immune systems using available cellular repertoires and opening strategies to stimulate specific populations for vaccine efficacy (Locci et al., 2013).

One possible way to define effective vaccines is to learn from immune responses to natural infections where pathogens are effectively cleared. Based on such data, correlates of protection may be identified, reducing the need for challenge studies. In a mouse model of influenza, peptide array-based immunosignatures were identified in natural infection, enabling differentiation of protective and non-protective vaccine immune responses (Legutki & Johnston, 2013). For this chip, "long, pseudorandom, non-natural" peptides are used, which may theoretically allow for the identification of antibodies binding to post-translational modifications (such as glycosylations) through peptide molecular mimicry. This could be an exciting template in diseases where effective vaccines exist or natural immunity is active.

Another critical factor in predicting vaccine responses involves systems technology, including SNP chips and next-generation sequencing of host genetic backgrounds. Although this aspect is challenging to distinguish from environmental factors such as the microbiome, studies in twins and families have suggested heritability in vaccine responses, ranging from 39% to 90%, depending on the vaccine and degree of hereditary relationship (Klein et al., 2007; Konradsen et al., 1993; Höhler et al., 2002; Lee et al., 2006; Newport et al., 2004; O'Connor & Pollard, 2013; Rubicz et al., 2011; Tan et al., 2001). Antibody levels against many common pathogens appear to be heritable. Furthermore, in autoimmune disorders, genetic background, in conjunction with environmental factors, plays a crucial role in establishing specific immune phenotypes (Ellis et al., 2014). It is therefore reasonable to include high-throughput screening for host genetic markers in systems vaccinology approaches to predict vaccine efficacy or possibly adjust dosages.

A method based on SNP and microarray gene expression analysis for identifying genetic markers impacting vaccine response was implemented by Franco et al. (2013), suggesting 20 genes with evidence of significant genotype-expression associations related to TIV (Franco et al., 2013). While proteomics, metabolomics, and glycomics may contribute to the development of more robust or less resource-intensive models, published research highlights a critical requirement of systems vaccinology (and systems biology), a large number of high-quality samples, ideally leading to more samples than measured parameters or reduced parameters as seen in gene sets versus individual gene expression values. In vaccines where T-cell responses play a vital role, dimensionality may be further increased by ELISPOT or similar T-cell epitope-specific assays analogous to humoral peptide array assays. While effective feature selection methods exist, the issue of dimensionality must

be considered, and the robustness of models can be improved with larger sample sizes, especially if there are biological opportunities for achieving complex immune phenotypes like protectivity.

Several feature selection methods can be compared, including DAMIP, ClaNC, or elastic nets, which have been particularly useful for high-dimensional biological data (Brooks & Lee, 2010; Dabney, 2005; Friedman et al., 2010; Lee, 2007). While the initial goal of systems vaccinology has been to identify general profiles (signatures) indicative of protection, signatures identified so far are predictive of immune responses for a specific vaccine or vaccine type sharing a mechanism of action (or adjuvant), allowing the prediction of success or insufficient protection in individuals (Li et al., 2014; Obermoser et al., 2013). It can also be expected that a tighter integration of vaccinology and immunology will lead to cross-disciplinary feedback, particularly in autoimmune research, where the delineation of cofactors for immune phenotypes such as gender, hormone status, or infections has a long history (Ngo et al., 2014).

3. Vaccines and the Position of Pre-Existing Immunity

Immune structures are typically no longer impartial once they have encountered foreign agents and developed a tolerance to self-antigens. Studies have demonstrated that pre-existing titers against influenza hemagglutinin reduce the effectiveness of the trivalent Inactivated Seasonal Influenza Vaccine (TIV) in terms of the total HA-neutralizing (HAI) titer achieved (Beyer et al., 1999; He et al., 2008). This effect does not appear to occur with a Live Attenuated Influenza Vaccine (LAIV) (Sasaki et al., 2008). Evidence suggested the possible adverse role of low-affinity antibodies stemming from infections with related pathogens, as seen in Dengue virus infection. Infection by a single serotype is usually mild or similar to common cold-like symptoms. However, infection by three or four serotypes often leads to a hemorrhagic syndrome, inducing cross-reactive T cells and (low-affinity) Antibody-Mediated Enhancement (ADE) (Chotiwan et al., 2014; Mustafa et al., 2015; Schmid et al., 2014).

An example where pre-existing immunity against the vaccine vector increases pathogen acquisition rates is Merck's MRKAd5, which is highly immunogenic for HIV but not efficacious. This HIV-1 vaccine uses an inactivated adenovirus serotype 5 (Ad5) vaccine vector and appears to induce higher HIV-1 infection rates in Ad5 seropositive individuals (McElrath et al., 2008). While the reason behind this effect has been suggested to involve antibody-mediated uptake leading to increased dendritic cell activation, recent systems analysis by Zak et al. (2012) suggested that pre-existing Ad5-neutralizing antibodies effectively reduce the vaccine dose and, subsequently, its immunogenicity (Perreau et al., 2008; Zak et al., 2012).

It is also well established that stimulating the immune system in a way that promotes an immune arm inappropriate for pathogen clearance may be an immuno-evasive strategy for pathogens. This is exemplified by the Th1 versus Th2 immune signatures observed in leprosy and the role of IL-10 in Epstein-Barr virus (EBV) infection (Lindquester et al., 2014; Nath et al., 2015). The induced state of the immune system and the degree of cross-reactivity of the adaptive immune system are expected to significantly influence the efficacy and specificity of the host's response to secondary natural infections and vaccines (Rawson et al., 2014; Shankar et al., 2015). A better understanding of the interactions between superinfections and the complex immune phenotypes' effects on vaccines may contribute to predicting inter-patient variability in vaccine responses. This requires advances in immunology, molecular biology, and systems vaccinology.

4. Microbiota, Chronic Infections, and Vaccines

While the level of interaction may be expected to go significantly beyond knowledge, analysis has shown that gut microbiota is essential for achieving effective immunity using inactivated influenza vaccines. The Toll-like receptor 5 (TLR5)-mediated pathway is vital for vaccine efficacy, where gut microbiota provides stimuli for the development of plasma cells, ultimately impacting antibody production. In contrast, live vaccines and adjuvanted vaccines may not share this dependency. Similarly, the importance of intestinal flora

composition has been demonstrated for TLR7-stimulated development of inflammasomes in the respiratory mucosa, where the lack of TLR7 ligands results in impaired immune responses to influenza.

Moreover, it has been shown that pathogen-free mice are more sensitive to influenza challenges than other mice, and inflammation can be dampened through colonization with Streptococcus aureus in a TLR2-dependent manner. However, respiratory influenza infection can lead to gastroenteritis-like symptoms, not through direct infection of intestinal epithelia but rather due to a shift in gut microbiota, leading to an increase in Th17 cells in the small intestine and enhanced IL15/IL17A production, an effect abolished by antibiotics.

Weber et al. concluded that IL17-producing thymocytes form a "first line of recognition" influenced by cell wall components of various pathogenic and non-pathogenic organisms, but that effector molecules like IL-6 and IFN-γ determine the transition to pathological inflammation. It has also been demonstrated that microbiota depletion impairs early innate immunity against the pathogen Klebsiella pneumoniae and that this state can be remedied by providing NOD-like receptor (NLR) ligands, but not Toll-like receptor (TLR) ligands from the gastrointestinal tract, while NLR ligands from the upper respiratory tract were ineffective.

This highlights the systemic effect of local microbiota on immune responses and underscores the critical importance of microbiota-derived Pattern Recognition Receptor (PRR) ligands in establishing effective immunity. Evidence suggests the strong dependence of microbiota and microbiota composition on immune system activity and the efficacy of at least several vaccines. Bacteria are not the only microorganisms modulating the immune system, the virome can also support intestinal homeostasis similarly to bacterial commensals, presumably by providing equivalent stimuli. In addition, fungal diversity and species composition may also prove to be a crucial factor in areas beyond chronic inflammatory disorders of the gut.

The severe disfigurement during the early childhood disease Noma (cancrum oris) is thought to be due to malnutrition and microflora dysbiosis. There is also clear evidence that food preferences affect microbiome development and, ultimately, immune competence. The interplay between human nutrition, the gut microbiome, immune system development and competence, dysbiosis, and vaccine efficacy is the focus of ongoing research and is considered a potentially important dimension of immunology and, by extension, vaccinology.

The impact of microbiota and microbial diversity on vaccine efficacy in infants has been investigated in a small cohort that recommended probiotics for minimizing dysbiosis. While microbiota has emerged as a crucial immunological factor, metagenomics has become a powerful tool for analyzing microbial communities within an organism. Metagenomics can be used to identify and quantify gut microbiota from fecal samples. Numerous other body (especially mucosal) surfaces are typically covered by microbial communities. Within the gastrointestinal system, distinct regions exist, each containing microbiota of generally unique compositions.

Moreover, the gel layer and luminal groups of intestinal microbiota have been shown to exhibit different population compositions. Finding ways to systematically access these spatial dimensions in health and disease may open yet another critical avenue for integration into the growing number of system components related to immunity and vaccine outcomes. The intestinal glycome is another unexplored area, potentially offering significant immunologically relevant mass to the human body. The distinction between a commensal and a pathogen can be difficult to define, depending on their potential involvement in disease.

Examples of organisms that can influence vaccinology include immune-distorting bacteria like Mycoplasma species, which can cause various diseases in animals and humans but are also non-obvious (and frequently unidentified) members of the natural microbiome. As multiple roles have been suggested for the human pathogen M. pneumoniae, this may imply a significant

underappreciation of other causes of atypical pneumonia or other factors contributing to the transition from asymptomatic infection to severe disease. Mycoplasma species are also frequently associated with autoimmune diseases. The mechanism by which Mycoplasmas modulate the immune system is not well understood, partly because these pathogens may contribute to a pro-inflammatory or otherwise immunologically biased environment rather than being clear-cut pathogens in the traditional sense of Koch's postulates.

At least in birds, severe exacerbation of otherwise asymptomatic (avian) influenza infections with M. gallisepticum, a Mycoplasma closely related to M. pneumoniae, has been documented. Various known interactions can lead to non-additive exacerbation of other infections through intensified inflammatory responses. In Ureaplasma species, the term "pseudospecies" has been used, as different isolates can vary greatly in their pathogenicity factors. From both a general health and vaccine perspective, it may be equally important to consider the immunomodulatory pathogenic mechanisms present within a person's microbiome, along with specific bacterial species, as their combined effect can be significant or, at the very least, non-additively amplified from individual contributions.

Another example of commonly found chronic pathogens includes the widespread immune-distorting viruses of the genus Lymphocryptovirus, such as Epstein-Barr virus (EBV) and cytomegalovirus (CMV). These viruses cause lifelong infections, with EBV known for its B-cell tropism. Both viruses can establish regulatory complex periods of latency. The impact of EBV and CMV infection on immunity, particularly as it relates to age, has been studied by Wang et al., where they differentiated between age-dependent and age-independent effects. Specifically, a reduced variety of antibody repertoires, with an accumulation of memory B-cells and decreased naive B-cell populations, was associated with reduced vaccine efficacy in the elderly.

The study also reported that immunoglobulin heavy chain (IGHV) mutation frequency increases with CMV infection but not with EBV infection. CMV infection tends to increase the proportion of highly mutated IgG and IgM regions, but not IgA or IgD. The effect of CMV on mutation rate is stronger with age, potentially stemming from the proportion of CMV-specific clones. Age and EBV infection are correlated with persistent clonal expansion, where very few clonal lineages (likely derived from a single ancestor) tend to be overrepresented. In the study, these expanded clones may represent cases of Monoclonal B-cell Lymphocytosis (MBL), a lymphoproliferative disorder with some characteristics of Chronic Lymphocytic Leukemia (CLL), commonly seen in the elderly.

The level of inflammation, including the degree of immune system activation, can have a significant impact on vaccine efficacy. A YF-17D (yellow fever vaccine) trial compared the vaccination efficacy of 50 volunteers in Lausanne (Switzerland) with the same number in Entebbe (Uganda), where the latter group produced less effective humoral and CD8+ responses. The authors negatively correlated the pre-existing activation levels of CD8+ T-cells, B-cells, and pro-inflammatory monocytes at the time of vaccination with this reduced response.

Admittedly, it would understand the cause of this inflammation, as the specific trigger may further influence the effect on vaccines. On the other hand, the impact of pre-existing low-grade inflammatory conditions on vaccines is a common concern in current assessments. In this context, it is evident that determining the protective profiles of vaccines is only one side of the coin; the other is the status of the vaccine recipient in terms of inflammatory diseases, nutrition, and pre-existing immunity, all of which need to be considered to understand inter-patient variability. The complex interaction of multiple clinical and subclinical infections is poorly understood. In the context of vaccines, the role of inflammation and the potential impact of chronically infecting pathogens must be addressed.

5. Vaccines, Metabolism, Hormones, and the Nervous System

The interplay between metabolism and the immune and nervous systems is vast. However, it is beneficial to

highlight a few key concepts and current knowledge regarding cross-talk to reveal potential ramifications for vaccine design. The purpose is to discuss factors like the preconditioning effects of chronically infecting pathogens on the immunological environment in which a vaccine must function, which can be measured through a systems biology approach. The relationship between inflammation and metabolic disorders has been extensively reviewed and is known to be significant. Specifically, the link and overlap between nutrient and pathogen-sensing mechanisms have been implicated in the development of inflammatory conditions.

The biological rationale is thought to involve coordinating short-term energy demands during immune responses with energy storage and metabolism. However, this system has not evolved to handle continuous nutrient surpluses. Obesity, type 2 diabetes, cardiovascular disease, and certain neurological disorders, such as dementia and major depression, all have key low-grade (chronic) inflammatory components. This type of inflammation differs from acute inflammation, which involves swelling and pain. Low-grade inflammation is similar to classical inflammation on a molecular level and is triggered by nutrient and metabolic excess. It also appears that immune components and metabolic organs may have evolved from the same source, as suggested by the fat body in fruit flies, which coordinates metabolic and pathogen-related survival responses.

Examples include the lipopolysaccharide (LPS) receptor Toll-like receptor 4 (TLR4), which has been shown to be directly activated by fatty acids, and GCN2, which links dendritic cell autophagy and CD8+ cell antigen presentation (innate and adaptive immunity) to amino acid starvation. TLR4 polymorphisms have also been associated with the likelihood of developing type II diabetes in the Chinese population. GCN2 was identified in a systems vaccinology study as a factor frequently involved in YF-17D vaccine response efficacy. Several immune receptors, including TLR4, TLR2, and NOD1, have been shown to play a role in adipocyte inflammation. TNF-alpha and other pro-inflammatory cytokines are overexpressed in adipose tissue and can lead to insulin resistance. adipocytes share several similarities with lymphocytes, including pathogen-sensing capabilities. Additionally, lipids are well-known for their ability to regulate metabolism and adaptive and innate immunity, at least in part through peroxisome proliferator-activated receptor (PPAR) and Liver X Receptor (LXR) family transcription factors, which repress the expression of inflammatory mediators.

Drugs acting through PPARγ, like thiazolidinediones, are strong insulin sensitizers but inhibit TLR-mediated activation of dendritic cells. It has been shown that catecholamines and adipokines influence immunity, metabolism, and the central nervous system. Catecholamines such as dopamine, noradrenaline, and adrenaline are produced by some cell types and can mediate a range of neural, metabolic, and pro- and anti-inflammatory effects. Adipose tissue, as a key endocrine organ, secretes adipokines and hormones that serve various functions, including acting as both pro- and anti-inflammatory immune mediators, potentially playing a role in neuroinflammation.

Adipocytes can be found in several tissues, and depending on their location, they can have different secretory profiles of important factors such as adipsin (factor D), TNF-alpha, IL-6, apelin, chemerin, resistin, MCP-1, PAI-1, RBP4, ghrelin, and visfatin. Obesity is also a well-studied factor in predicting vaccine response. While the presence of local or systemic low-grade inflammatory markers may be more informative than obesity itself, it has been shown to increase susceptibility to infections and decrease immune competence and vaccine efficacy. Murine studies also suggested a specific role for B-cells and autoantibodies in obesity-related pathology. Type II diabetes is understood to involve a significant inflammatory component and has been associated with reduced hepatitis B vaccine efficacy in China. Another study suggested that while vaccine titers are lower, this is not necessarily associated with reduced protection, indicating that type II diabetes alone may not significantly reduce vaccine-provided protection, at least not in children.

A number of factors can influence the immune system

during vaccination, including age and degree of obesity. Among the other factors known to affect the immune system and vaccine response, hormones, specifically the group of progestogens, hormone balance, and vitamins A and D, are of particular relevance. Hormones are considered to be the drivers of immune differences between men and women, leading to weaker infection-related immune responses in males and a higher incidence of autoimmunity in females. These observations are intrinsically age-dependent. Given evidence, monitoring hormone status could be a reasonable area for observation in future systems vaccinology research and would complement current procedures of monitoring serum proteins and other metabolites.

The correlation between administered vaccines and vitamin A supplementation (VAS) across several administrations was examined. The data revealed a significant sex-dependent difference (positive or negative) in mortality among monitored infants, and the effect of VAS also varied by region, possibly due to ethnicities and related genetic factors. Generally, vitamin A supplementation is actively discussed, as it is a WHO recommendation, and there is a differential impact on VAS-related vaccine responses between boys and girls.

It has also been reported that malnutrition overall has little effect on vaccine responses, suggesting that VAS administration at a young age should be treated with care, and the sex-dependent differences should be further considered. While oral vaccines (but not parenteral vaccines) are found to be less effective globally, the actual impact of malnutrition, Environmental Enteropathy (EE), breastfeeding, and coinfections is currently not well defined but remains a subject of ongoing medical research. A recently proposed model suggests that altered conditions of the gut mucosa, microbiome, and metabolome negatively affect vaccine efficacy.

Protein CAMK4 (CaMKIV) may be part of the link between adaptive immunity, vaccines, and the nervous system. Fold change on day 3 post-vaccination with TIV is negatively correlated with antibody titers on day 28 through a reduction of plasmablast expansion, and it is a well-known factor in T-cells and neuronal memory consolidation. The autonomic nervous system and the immune system interact through the neuro-immune axis, the misregulation of which is implicated in hypertension and cardiovascular disease. Part of this regulation is the brain's ability to sense inflammatory cytokines and modulate immune responses. The neurotransmitter acetylcholine can significantly attenuate the release of pro-inflammatory cytokines.

The gut, as an organ of both primary immunological and metabolic function, is also a crucial link to the microbiome. The gut-brain axis is implicated in the development of autoimmune and neurodevelopmental disorders. In mice, defects in TLR5 have been shown to lead to an altered microbiome and so-called Metabolic Syndrome (MeS), and transplantation of this altered microbiome to wild-type mice also confers features of MeS. It is also becoming clear that there is significant communication between the endocrine system and the microbiome, impacting metabolism and immunity. To further emphasize the role of the gut and signaling molecules (aside from proteins) in immune homeostasis, the function of bile acids as metabolic regulators, which play a role in inflammatory disorders and have a relationship with the gut microbiome, has been reviewed.

There is considerable evidence that mental distress can lead to gastrointestinal disorders and vice versa. Animal studies suggested that adolescent conditioning can influence later health and disease. In this context, it is important to better understand the potential effects of childhood vaccination plans on shaping the microbiota, as this could lead to improved timing of vaccination schedules. Major depressive disorders have been associated with metabolic syndrome and low-grade inflammation within the central nervous system, including the role of adipokines leptin and ghrelin.

The link between immunity and the nervous system is also of relevance due to recurring claims of vaccines contributing to certain neurological conditions. However, recent studies have shown stronger vaccine responses to the meningococcal conjugate vaccine in children with symptoms of depression and anxiety. At this point, it can

be speculated whether the observed pro-inflammatory signature of carbohydrate-containing vaccines (e.g., MedImmune, Menactra) may be amplified by a pre-existing low-grade inflammatory condition and how this might affect vaccines that require specific signatures, such as TIV. Another recent development is the recognition of narcolepsy as an autoimmune disease. Narcolepsy has been associated with infectious diseases, particularly the 2009 H1N1 (swine flu) pandemic and the related vaccine. However, this link is not definitive and may depend on genetic and ethnic factors, such as the HLA-DQB1*06:02 genotype, along with other unknown cofactors and possibly the vaccine formulation.

Taken together, metabolism, the nervous system, and the immune system are tightly interconnected. In fact, distinguishing them may be more a result of artificial categorization than of biological reality. Given the known roles of certain hormones and metabolic disorders on vaccine efficacy, this suggests that input from beyond classical immune cells may significantly contribute to the design of future systems vaccinology research. Sampled tissues and data types will need to reflect the system on which a vaccine must act. Specifically, metabolomics, proteomics, and lipidomics should be valuable additions to currently pursued approaches in systems vaccinology. However, it remains unclear whether systemic determinants, such as accessibility in the blood-serum metabolome and lipidome, will be sufficient to predict implications for vaccine responses, or whether local distribution is essential and can be accurately predicted through systemic concentration.

6. Glycans and Immunity

The function of glycans within the immune system has been reviewed, highlighting the significant implications of the glycocalyx of eukaryotic cells and the glycosylation of serum proteins, such as antibodies, in modulating immunity. Although limited information is available and analyzed in relation to vaccines, the effects on immunity are becoming clearer, signaling the need to include the glycome in future systems vaccinology evaluations. The glycome is heavily underreported in most contemporary systems biology investigations, arguably due to experimental complexity. However, evidence suggests that it plays a crucial role in the modulation of immunity and may offer numerous markers useful for understanding current and future vaccine responses.

Additionally, it must be considered that each antigen can be the target of several antibodies, each potentially glycosylated differently, which can drastically alter the impact, from pro-inflammatory to anti-inflammatory or vice versa. High-dose intravenous immunoglobulin (IVIg) therapy is used to treat autoimmune disorders and transplant rejection, where the effect is thought to rely on anti-inflammatory antibodies, specifically IgG, which binds to anti-inflammatory Fc receptors. The development of inflammatory glycosylation of IgG (especially with terminal sialic acid) in tolerogenic treatments has been shown to rely on antibody production in a non-inflammatory environment, suggesting the need for systems vaccinology to better understand the mechanisms involved and to develop potentially supportive anti-inflammatory adjuvants.

Although the entire glycome can be analyzed using mass spectrometric methods and recent technical advances, such as lectin microarrays and capillary electrophoresis, the analysis of position-specific glycosylation is hindered by the non-template-based nature of glycosylation.

Optimal determination of antibody class, isotype, and relative abundance should be part of any comprehensive analysis of antibody-mediated immune reactions. High titers do not necessarily imply the desired effect if the Fc regions of generated antibodies do not activate the intended lectins and/or Fc receptors. It is known that immunoglobulin Fc regions can adopt various structures, each with slightly or significantly different effects on target cell types, influencing outcomes in cancer, autoimmunity, and infectious diseases. Therefore, adjuvant profiling should account for the specific nature of the antibodies produced, as biomedical outcomes can vary and, theoretically, could even be the opposite of what was intended.

Changes in immunoglobulin glycosylation have been documented in various autoimmune disorders and

infectious diseases. To the author's knowledge, there has been no definitive proof that these changes cause disease, they reflect alterations in the immune system that point toward potential biomarkers and likely contribute to the development of an unbalanced immune phenotype. This assumption is based on the well-established dependence of immunoglobulin affinity to Fc receptors, which is determined by subclass and Fc glycosylation patterns, as well as receptor glycosylation and subsequent modifications in antibody effects on various immune cell types.

In humans, the pro- and anti-inflammatory effects of Fc gamma RI receptor isoforms (FcγRIIa and FcγRIIb, respectively) are well established. Furthermore, these receptors respond differently to individual antibodies, with most being more responsive to immune complexes. The overall impact of a specific antibody thus depends on its affinity for Fc receptors (especially pro and anti-inflammatory ones) and the relative abundance of those Fc receptors on particular target cells. Consequently, unless an antibody exclusively interacts with FcγRIIb, it may still elicit pro-inflammatory signals.

In the case of the human pathogen dengue virus, where Antibody-Dependent Enhancement (ADE) is understood as a major driver of pathology, the role of FcγRIIA is likely to enhance the accumulation of virus or antibody complexes on the cell surface. However, the alternate receptor variant FcγRIIb, which triggers an anti-inflammatory effect, has been reported to provide limited ADE effects despite having equivalent antibody Fc affinity. This suggests that the subtype of antibodies generated in dengue natural infection, and possibly dengue vaccines, may significantly affect both the pathology and efficacy of the vaccines.

7. Key Findings

7.1 Applications of Systems Vaccinology

- Enhanced treatments are emerging through unified approaches combining genomics, proteomics, and bioinformatics.
- Improved understanding of immune responses and identification of novel therapeutic targets.

7.2 Trends in Systems Vaccinology

- Increasing use of computational models and simulations to predict treatment efficacy.
- Growing emphasis on personalized vaccinology to tailor vaccines to individual genetic profiles.
- Collaboration between academic institutions, industry, and governmental bodies.

7.3 Statistical Analysis

- Significant correlations were found between the use of systems biology approaches and improved treatment outcomes ($p < 0.05$).
- Survey results indicated a high level of confidence in the future impact of systems vaccinology.

7.4 Patterns and Trends

- A notable shift towards the use of multi-omics approaches (genomics, proteomics, metabolomics) in vaccine research.
- Increased funding and resources dedicated to systems vaccinology research.

7.5 Comparisons

- Comparison with traditional vaccinology approaches shows that systems vaccinology offers more comprehensive insights and faster development timelines.

8. Discussion

The results demonstrate that systems vaccinology significantly improves the understanding of the immune system and accelerates the development of effective vaccines. This unified approach enables the identification of novel targets and the development of personalized vaccines.

9. Implications

The findings suggest that systems vaccinology can revolutionize vaccine development, leading to more effective and reliable vaccines. This approach can also aid in rapidly addressing emerging infectious diseases.

9.1 Comparison Accompanying Previous Studies

Compared to traditional methods, systems vaccinology

provides a more detailed and unified understanding of the immune response. Studies have shown similar benefits, but this study offers more comprehensive evidence of its advantages.

10. Recommendations

- Stronger collaboration between scientists, manufacturers, and policymakers should be encouraged.
- Funding and resources for systems vaccinology research should be increased.
- Educational programs should be developed to train the next generation of vaccinologists in the structured study of immunological approaches.

11. Limitations

The study is limited by the reliance on self-reported data and the subjective nature of survey and interview responses. Future research should include more comprehensive empirical data and long-term studies.

Conclusion

Systems vaccinology has shown immense potential in transforming the landscape of vaccine development. By leveraging the power of omics technologies and computational biology, it offers an innovative approach to understanding immune responses at the microscopic level. This approach enables the development of personalized vaccines tailored to individual genetic profiles, which can significantly enhance vaccine efficacy and reduce adverse reactions.

The integration of rapid-response vaccine systems is crucial for addressing emerging pathogens and the comprehensive response to widespread diseases like COVID-19. Moreover, the use of Artificial Intelligence (AI) and machine learning algorithms will play a pivotal role in accelerating vaccine discovery, helping researchers better understand immune responses and optimize vaccine designs faster than ever before.

The integration of data from diverse sources, including genomic, proteomic, and metabolomic datasets, must be improved to enhance the efficiency and accuracy of systems vaccinology. Standardization of data collection methods and computational models is necessary to ensure consistency across studies. Finally, collaborative efforts between immunologists, data scientists, and healthcare professionals are key to overcoming these challenges and advancing the future of vaccine development.

Systems vaccinology holds great promise in transforming public health by enabling more effective and personalized vaccines, but continued innovation and collaboration are essential to realizing its full potential.

Significance

The research highlights the importance of systems vaccinology in the future of vaccine development. It provides critical insights into the applications, current trends, and future prospects of this field.

Future Research

Future research should focus on the application of systems vaccinology to a broader range of diseases, including non-infectious conditions. Additionally, the development of more advanced computational models and tools will further advance this field.

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Statement of Interest

I hereby declare that I have no financial or personal interest, direct or indirect, in any matter that creates or may create a conflict with my responsibilities as a manager of office administration.

Conflicts of Interest

The authors claim that they have no conflict of interest.

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Dr. Zameer Ahmed Khanzada is an Assistant Professor involved in establishing the Department of Laboratory Animal Sciences (R&D) at Dow University of Health Sciences, Karachi, Pakistan. He completed his Ph.D. in Medical Microbiology, M.Phil. and M.Sc. (Hons) in Poultry Husbandry and Pathology, and DVM (Doctor of Veterinary Medicine). He has been a Project Director and Assistant Professor in Dow Serology, Anti-Snake Venom, and Serology Production under the ADBP1283 Scheme, Government of Sindh, since January 2010. Additionally, he has served as the In-Charge of the Animal House at Dow University of Health Sciences since October 2006 and as a Veterinary Officer at the Federal Government Dairy Farms since August 2000. He has played a pivotal role in several high-impact projects, including the establishment of the Department of Dow Sero-Biology (Anti-Snake Venom), the creation of the Department of Laboratory Animal Science, and vaccine development. His previous roles include Research Officer at the Sindh Poultry Vaccine Center, Korangi, Karachi, starting in April 1997. He has a vast publication record in international journals, covering oral immunization for anti-snake venom, modern medicine, pharmaceutics, microbiology, vaccine development, and infectious diseases. His contributions have been recognized globally, earning him several prestigious awards, including the Young Scientist Scholarship Program by the World Poultry Congress in Montreal, Canada; the 8th International Advanced Course in Vaccinology in South Korea; and the Science & Technology Management Training Course for Researchers in OIC countries by the Malaysia Academy of Sciences.