Chapter 5

Systems Vaccinology: Applications, Trends, and Perspectives

Johannes Sollner

Abstract

The strategies employed in vaccinology have improved since the seminal work of Edward Jenner in the eighteenth century. Stimulated by failure to develop vaccines for cancers and chronic infectious diseases as well as an emergence of a multitude of new technologies not available earlier, vaccinology has moved from a largely experimental art to a new phase of innovation. Currently, immune reactions can be predicted and modeled before they occur and formulations can be optimized in advance for genetic background, age, sex, lifestyle, environmental factors, and microbiome. A multitude of scientific insights and technological advancements have led us to this current status, yet possibly none of the recent developments is individually more promising to achieve these goals than the interdisciplinary science of systems vaccinology. This review summarizes current trends and applications of systems vaccinology, including technically tangible areas of vaccine and immunology research which allow the transformative process into a truly broad understanding of vaccines, thereby effectively modeling interaction of vaccines with health and disease. It is becoming clear that a multitude of factors have to be considered to understand inter-patient variability of vaccine responses including those characterized from the interfaces between the immune system, microbiome, metabolome, and the nervous system.

Key words Systems vaccinology, Systems biology, Vaccine, Metabolism, Prediction, Signature, Microbiome, Metabolome, Immunogenicity, Protectivity

1 Introduction

Systems vaccinology (SV) is a nascent science which has emerged as a variant of s*ystems biology* and aims to understand the effect of vaccines on the entire host system. In contrast to systems biology, SV is therefore heavily application focused, and possibly only in that respect different from the more generally used term *systems immunology*. Like all areas of systems biology, the aim of SV is to overcome reductionist thinking and generate a more comprehensive, dynamic, and in a way more realistic understanding of the interaction of components in an organism, in this specific context of reactions induced by vaccines or factors influencing efficacy of vaccines. While this is not a new concept, viewing the host as a

Sunil Thomas (ed.), *Vaccine Design: Methods and Protocols: Volume 1: Vaccines for Human Diseases*, Methods in Molecular Biology, vol. 1403, DOI 10.1007/978-1-4939-3387-7_5,

system interacting with the environment (also in many cases a set of interacting systems) allows identification of signatures and interactions critical for defining or achieving states critical for success of a therapy, drug, or vaccine. These signatures may not be evident from a reductionist point of view, at least not without complete deconvolution of all possible components. Being embedded in a complex system, understanding host components and their interaction with environment in a single picture is critical for rationally designing drugs including structure-based vaccines. This is particularly important in the case of chronic infections where the system constituting an intruding pathogen establishes a continuous but unfortunately not mutually beneficial relationship with the host system. Understanding host/pathogen interfaces will therefore be another important area within systems vaccinology and may very likely break the boundary to other areas of pharmacology to further expose and target this host/pathogen interface. Chronic infections are typically established by a pathogen by mechanisms which make host defenses at least partially ineffective by remodeling host immunity as well as by immunological stealthiness. Likewise, cancer vaccines (with few exceptions) to date have not been very successful. Understanding the complex interaction of cancers with their environment (including tumor stroma) employing the concept of SV will facilitate effective cancer vaccines. While vaccines have been tremendously successful, the modes of action of a number of commonly used vaccines are not well understood. Infectious diseases, including HIV, malaria, tuberculosis, and dengue fever, have proven to be at least partially resistant to traditional vaccination approaches. Systems vaccinology offers powerful tools to monitor a multitude of data types sampled in parallel through omics technologies to reconstruct a comprehensive picture of processes leading to success or failure of a vaccine or a vaccine prototype. As recently reviewed by Pulendran, Rappuoli, and others, SV therefore primarily offers a deeper understanding of processes leading to or prohibiting protective immunity and establishes a basis for vaccine developers to overcome old limitations [1, 2]. This approach of understanding vaccines will become even more complex as sources of variation between immune responses become more evident and tangible, specifically genetic host diversity, environmental and psychological factors, impacts of the microbiome, and socioeconomic factors including associated disorders such as obesity, diabetes, and those resulting from malnutrition. Systems vaccinology offers a way to gain deeper understanding of the ongoing pathomechanisms by comparing to successful immune responses, thereby leading to the development of new immunization strategies for diseases that do not yet have a commercially successful vaccine. Successful vaccines that induce the immune system can be modeled based on expression signatures or molecular

processes that clear pathogens during an infection. Data from entire populations as well as nonresponders could help in designing vaccines. The type and intensity of immune reactions can among other things be effectively modulated by pattern recognition receptors (PRRs) including Toll-like receptors (TLRs) and nucleotide-binding oligomerization domain (NOD) proteins, which constitute some of the most effective adjuvant targets known. Understanding the immune-evasive mechanisms as well as the latest immunological insights and mechanisms of novel adjuvants will lead to reprogram ineffective to effective immunity.

Some currently pursued and further perceivable applications of systems vaccinology are listed below:

1. Predict immunogenicity and optimal protectivity of vaccines in an individual based on host samples taken before or after vaccination. Applications include stratification of patients for clinical trials, early identification of nonresponders which may allow application of alternative medications, and risk assessment regarding adverse effects.
2. During vaccine research, taking comprehensive immunological snapshots should speed up development, reduce the risk of failures in later clinical trials, and improve understanding of immunity in risk groups including the very young and the elderly. Specific identification of novel formulations and adjuvants, ex vivo (peripheral blood mononuclear cell (PBMC)) optimization of dosage, relative and absolute adjuvant content, and better understanding of the relationship of immunity with metabolism and neuronal system are required.
3. Another goal is to understand the interface of vaccines with complex phenomena like cancer and autoimmunity, including autoantibody levels and antibody subclasses. Understanding the potential of vaccines to modify diseases with inflammatory components such as neoplastic disorders, stroke, and coronary heart diseases may have drastic beneficial effects on public health. This extends to the idea to use self-associated molecular patterns (SAMPs) to reduce inflammation, an option which seems tangible due to insights into the glycobiology of antibodies including anti-inflammatory effects of targeted Fc receptors.

2 Understanding and Prediction of Vaccine Responses

The most frequent application of systems vaccinology to date has been the definition of critical parameters associated with immunogenicity and optimal protectivity of a vaccine. The aim of a number of studies has been to define systems signatures, specifically based

on gene expression analysis, which can be used as surrogates for vaccine efficacy. Application of these signatures would allow:

1. Evaluation and optimization of vaccine candidates including choice of adjuvants or dosage based on observation of signatures in PBMCs in vitro.
2. Early identification of nonresponders after vaccination.

Meanwhile the concept has been extended to include features of the host before vaccination. A number of molecular and other properties have been identified to allow estimation of the vaccine response before treating a subject, which has so far been aimed for two related practical applications:

1. Stratification of patients for clinical trials based on likelihood to react to a vaccine.
2. Early identification of potential nonresponders before vaccination, which would allow selection of different vaccine products, dosage, or administration regimens.

Both the latter options are forms of personalized medicine. A third related option may 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 in a study of systems vaccinology where vaccinology and immunology were combined [3–6]. The initial study by Querec et al. succeeded to define and verify early post-vaccination gene expression signatures which predicted YF-17D adaptive immune responses, specifically $CD8^+$ (cytotoxic) T-cell responses with up to 90 % accuracy and the neutralizing antibody response with up to 100 % accuracy depending on evaluation procedure. This notably only requires models involving two to three parameters each. Given the wide use of this vaccine it may also be a good candidate for better understanding and prediction of adverse side effects including rare serious adverse effects [7–9]. Technologically this initial study has been shaping the conceptual approach to systems vaccinology until now. The authors combined FACS characterization of immune cells from patients sampled after vaccination in a time series, along 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) of the entire time series of fold changes. Results were corrected for multiple testing resulting in a set of 65 genes putatively differentially regulated in PBMCs upon vaccination. RT-PCR was used to validate differential expression of a subset of the indicated genes. For biological interpretation of microarray analysis results, differentially regulated genes were then subjected to gene enrichment analysis using DAVID to identify enriched pathways and modules [10]. In addition transcription factor-binding sites statistically

overrepresented in the promoters of these genes were identified using TOUCAN [11]. Obtained immune reactions were characterized by epitope-specific T-cell assays and determining neutralizing antibody titers. Interestingly the 65 differentially regulated genes were not useful (or insufficient) to predict magnitude of $CD8^+$ T-cell responses. For this purpose expression fold changes of individual genes at day 3 and separately at day 7 were individually tested for significant correlation with later $CD8^+$ T-cell response and neutralizing antibody response. In addition to PCA analysis which indicated a segregation of high and low $CD8^+$ responders, average linkage hierarchical clustering analysis was used and confirmed 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. have demonstrated that signatures of protectivity between live (LAIV) and inactivated (TIV) influenza vaccines vary where the authors specifically stress IFN-related genes as differentiating signature upon LAIV administration [12]. Similar to Querec et al. this study aimed to predict vaccine efficacy based on samples taken a few days after vaccination to allow prediction of hemagglutinin-inhibiting (HAI) titers a month later and specifically to allow identification of vaccine nonresponders as early a possible. While this study technologically also focused on gene expression analysis (microarrays and RT-PCR) and FACS they individually determined gene expression for PBMC subsets separated by FACS. This approach is highly reflective of the functionally differentiated nature of PBMCs and can lead to the identification of marker signatures otherwise invisible in bulk analysis due to expression primarily in critical but low-abundance subpopulations, demonstrated here by plasmacytoid dendritic cells (pDC). More cost- and logistically affordable analysis methods also capable of differentiating cell types have been proposed, specifically a meta-analysis procedure by Nakaya et al. and also statistical deconvolution using cell type-specific significance analysis of microarrays (CSSAM) [13]. In the case of Nakaya et al. meta-analysis suggested a TIV-specific pattern of highly expressed genes in antibody-secreting cells (ASCs), whereas cell types implicated in LAIV analysis were specifically T-cells and monocytes. These results show the importance of different cell types during analysis and how they modulate protective mechanisms during vaccination. A similar study of TIV without differentiating cell types was conducted by Bucasas et al. and also indicated a distinct gene expression signature comprising 494 genes segregating high and low vaccine responders [14]. Likewise, in a study conducted by Vahey et al. for prediction of protectivity of a malaria vaccine, the immunoproteasome and apoptosis were indicated as marker processes, depending on time after vaccination [15].

Furman et al. have strived to define markers of immunological health, where their idea was to use the intensity of immune reactions against an influenza vaccine as a measure of health [16, 17]. Notably, they identified nine features available before vaccination which predicted vaccine response with 84 % accuracy, plus a number of other parameters which also were individually predictive to some degree but did not further improve the model. Overall analyzed data included patient age, whole-blood gene expression profiles, peptide-specific anti-influenza antibody titer measured by peptide microarrays, 50 cytokines and chemokines, and typing to a resolution of 15 immune cell subtypes using FACS. The authors found that taken alone, age is the most informative predictor of HAI titers, where increased age correlated to reduced titers. As previously published by others, pre-existing HAI titers (for example stemming from previous vaccination or natural infection) are negatively associated with influenza vaccine-generated HAI titers, reportedly resulting from limited dendritic cell (DC) antigen presentation caused by pre-existing memory T-cells via natural killer (NK) cells. Furman et al. were able to specify a number of peptides which are predictive of this effect before vaccination and those that provide independent information to predict vaccine efficacy. In addition, whole-blood gene expression analysis followed by analysis of gene modules proposed a number of gene sets where expression was negatively correlated with post-vaccination HAI titers, and one associated with apoptosis which was positively correlated. The identification of apoptosis as critical parameter is reminiscent of the results described by Vahey et al. in malaria. The relevant information for predicting vaccine response was also present in levels of soluble Fas ligand (sFasL) and IL-12p40, and frequency of central memory $CD4^+$ and effector memory $CD8^+$ T-cells. From a number of perspectives also the setup of this study can be seen as exemplary for the identification of predictive models of vaccine efficacy, predominantly because of the number of omics techniques applied. Furman et al. observed (epitope specific) antibody titers based on peptide arrays, gene expression analysis of blood cells, and FACS-based immune cell subtyping. Independently, Tsang et al. have compared pre- and post-vaccination PBMCs using FACS and could show that pre-vaccination cell population frequencies alone are predictive of antibody response for TIV [18]. FACS-based discrimination of immune cell types followed by microarray analysis and cytokine panels has led to identification of $PD\text{-}1^+CXCR5^+$ $CD4^+$ T-cell numbers as indicators of emergence of broadly HIV-neutralizing antibodies. This population is similar to $CD4^+$ T follicular helper (Tfh) cells critical for B-cell maturation in germinal centers (GC), indicating the potential to characterize immune systems by available cell repertoire and opening strategies to stimulate specific populations for vaccine effect [19].

At least one of the possible ways to define effective vaccines is to learn from immune responses to natural infections where the pathogens are effectively cleared. Based on the data the correlate of protection may be identified, optimally reducing the need for challenge studies. At least in a mouse model of influenza, the peptide arraybased immuno-signatures have been identified in natural infection which allows differentiation of protective and non-protective vaccine immune responses by pattern [20]. For this chip "long, pseudorandom, nonnatural" peptides are used which may, at least theoretically, also allow identification of antibodies binding posttranslational modifications (including glycosylations) through peptide molecular mimicry. This may be an interesting template in diseases where effective vaccines exist or natural immunity is protective to serve as a template. Another important factor in predicting vaccine responses accessible to systems technologies includes SNP chips and next-generation sequencing of the host genetic background. Although this factor is very difficult to differentiate from environmental factors such as the microbiome, previous studies in twins and families have suggested heritability in vaccine responses to range between 39 and 90 % depending on vaccine and degree of hereditary relationship [21–28]. Interestingly, antibody levels against a multitude of common pathogens seem to be heritable. Similarly, in autoimmune disorders genetic background along with environment plays a crucial role in establishing specific immune phenotypes [29]. It is therefore reasonable to include high-throughput screening for host genetic markers into systems vaccinology procedures for prediction of vaccine efficacy or possibly adaptation of dosage. An approach based on SNP and microarray gene expression analysis for identification of genetic markers impacting vaccine response has been implemented by Franco et al. suggesting 20 genes that show evidence of significant genotype expression association related to TIV [30].

However while proteomics, metabolomics, and glycomics might contribute beneficially to the development of more robust or less resource-intensive models, the published studies show a critical requirement of systems vaccinology (and systems biology): a large number of high-quality samples, optimally leading to significantly more samples than measured parameters (which is admittedly unlikely), or possibly integrated parameters as seen in gene sets versus individual gene expression values. In vaccines where T-cell responses play a critical role, dimensionality can be reasonably further increased by ELISPOT or comparable T-cell epitope-specific data equivalent to humoral peptide array assays. While powerful feature selection procedures exist, the factor of dimensionality needs to be considered and robustness of models can be enhanced with larger numbers of samples, specifically if biologically alternative possibilities for achieving complex immune phenotypes

such as protectivity exist. Typically numerous feature selection methods will be compared, where some like DAMIP, ClaNC, or elastic nets have proven specifically useful for high-dimensional biological data [31–34].

While the initial hope of SV has been to identify general profiles (signatures) indicative of protectivity, signatures identified so far are predictive of immune responses for a specific vaccine or vaccine type sharing a mode of action (or adjuvanting), allowing to predict success or insufficient protection in individuals [35, 36]. It can also be expected that a tighter integration of vaccinology and immunology will lead to a feedback with related disciplines, specifically research into autoimmunity where delineation of cofactors for development of immune phenotypes including gender, hormone status, or infections has a long history [37].

3 Vaccines and the Role of Pre-existing Immunity

Immune systems are typically not neutral as they have previously encountered foreign agents and have built tolerance to self-components. Furman et al. and others have shown that pre-existing titers against influenza hemagglutinin reduce effectiveness of the trivalent inactivated seasonal influenza vaccine (TIV) in terms of total HA-neutralizing (HAI) titer achieved [38, 39]. This effect does not seem to exist for an influenza live vaccine (LAIV) [40].

Current evidence suggests possible detrimental role of low-affinity antibodies stemming from previous infections with related pathogens as demonstrated in *Dengue* virus infection. Infection by a single serotype is in most cases harmless, or comparable to infection as in common cold. However, infection by three or four serotypes frequently leads to a hemorrhagic syndrome, inducing cross-reactive T-cells and (low-affinity) antibody-mediated enhancement (ADE) [41–43].

An example where pre-existing immunity against the vaccine vector increases pathogen acquisition rates is Merck's MRKAd5/HIV which is highly immunogenic but non-efficacious. This *HIV-1* vaccine uses an inactivated adenovirus serotype 5 (Ad5) vaccine vector and seems to induce high *HIV-1* infection rates in Ad5 seropositive individuals [44]. While reason behind this effect has been suggested to be at least in part antibody-mediated uptake leading to increased dendritic cell activation, recent systems analysis by Zak et al. suggests that pre-existing Ad5-neutralizing antibodies effectively reduce dose of vaccine and hence immunogenicity [45, 46].

It is also well established that stimulating the immune system in a way as to promote an immune arm unsuitable for pathogen clearance can be an immuno-evasive strategy for pathogens, exemplarily demonstrated by the Th1 versus Th2 immune signatures seen in leprosy and the role of IL-10 in *Epstein-Barr virus*

(*EBV*) infection [47, 48]. The induced state of the immune system and degree of cross-reactivity of the adaptive immune system can be expected to severely shape efficacy and precise nature the host responds to secondary natural infections and also to vaccines [49–52]. Better understanding of the precise interaction of superinfections and interaction of complex immune phenotypes with vaccines may contribute substantially to prediction of inter-patient variability in vaccine responses. This calls for advances in immunology, molecular biology, and systems vaccinology.

4 Microbiota, Chronic Infections, and Vaccines

While the extent of interaction can be expected to go substantially beyond current knowledge, analysis has shown that gut microbiota are critical for achieving potent immunity using inactivated influenza vaccines. Toll-like receptor 5 (TLR5)-mediated pathway is critical for vaccine efficacy where gut microbiota provide stimuli for development of plasma cells ultimately impacting antibody production, while 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 respiratory mucosa, where lack of TLR7 ligands leads to impaired immune responses to influenza [53, 54]. Likewise, it has recently been shown that pathogen-free mice are more sensitive to influenza challenge than other mice and that inflammation can be dampened via colonization with *Streptococcus aureus* in a TLR2-dependent way [55]. However, respiratory influenza infection can lead to gastroenteritis-like symptoms not via direct infection of gut epithelia, but rather due to a shift in gut microbiota leading to increase in Th17 cells in the small intestine and also enhanced IL15/IL17A production, an effect abolished by antibiotics [56]. Possibly adding some detail to this observation, Weber et al. conclude that IL17-producing thymocytes form a "first line of recognition" stimulated by cell wall components of diverse pathogenic and apathogenic bacteria, but that effector molecules such as IL-6 and IFN-γ determine transition to a pathological inflammation [57]. 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 (NLR) receptor ligands but not Toll-like receptor (TLR) ligands from the gastrointestinal tract, whereas NLR ligands from the upper respiratory tract were ineffective [58]. This highlights the systemic impact of local microbiota on immune responses and suggests a critical importance of microbiota derived pattern recognition receptor (PRR) ligands for establishing effective immunity. In summary it can be stated that current evidence shows heavy dependency of microbiota and

microbiota composition on activity of the immune system and efficacy of at least some of the vaccines. Bacteria are not the only microorganisms modulating the immune system; the virome can support intestinal homeostasis comparable to bacterial commensals, presumably by providing equivalent stimuli [59, 60]. Similarly, fungal diversity and species composition may prove to be a critical extension also in other areas than chronic inflammatory disorders of the gut [61]. The terrible and disfiguring childhood disease Noma (cancrum oris) is currently thought to be caused by malnutrition and microflora dysbiosis [62]. There is also clear evidence that the choice of food impacts microbiome development and ultimately immune competence [63]. The interplay of human nutrition, gut microbiome, immune system development and competence, dysfunction, and vaccine efficacy is the focus of ongoing research and is now viewed as a very likely critical dimension of immunology and hence possibly also vaccinology [63–69]. The impact of microbiota and microbial diversity on vaccine efficacy in infants has recently been investigated in a small cohort by Huda et al. where they suggest probiotics for minimizing dysbiosis [70]. Of note in this context, while microbiota have emerged as an important immunological dimension, metagenomics has emerged as a powerful tool for analysis of the microbial community in an organism. Metagenomics could be used to identify and quantitate the gut microbiota of the fecal samples. Several other body (especially mucosal) surfaces are commonly covered by microbial communities; within the gastrointestinal system several distinct regions exist which contain microbiota of typically different composition. Moreover, the gel layer and luminal communities of gut microbiota have been shown to feature different population composition [71]. Finding ways to routinely access these spatial dimensions in health and disease may open yet another possible critical aspect for integration into the growing number of systems components regarding immunity and also vaccine effects. The gut glycome is another uninvestigated area presumably providing substantial immunologically relevant mass to the human body.

The distinction between a commensal and a pathogen can be difficult to draw, depending on the potential to be involved in disease. Examples of organism with potential impact on vaccinology are immune-distorting bacteria like Mycoplasma species which can cause diverse diseases in animals and humans [72] but are also non-obvious (and often unidentified) microbes of the natural microbiome [72, 73]. As multiple roles have been suggested for the human pathogen *M. pneumoniae* this may either mean a substantial underappreciation of other causes of atypical pneumonia or otherwise of other factors contributing to the conversion from an asymptomatic infection to a severe disease. Mycoplasma species are also frequently associated with autoimmune diseases [74]. The mechanism by which Mycoplasmas modulate the immune system are not clear,

and part of the reason is that these pathogens may contribute to a pro-inflammatory or otherwise immunologically biased environment rather than being clear-cut pathogens in the sense of Koch's postulates. At least in chicken severe exacerbation of otherwise asymptomatic (avian) influenza infection with *M. gallisepticum*, a Mycoplasma phylogenetically close to *M. pneumoniae*, has been documented [75]. Several known interactions exist where these can lead to nonadditive exacerbation of other infections through intensified inflammatory responses. At least in Ureaplasma species the term pseudospecies has been used, as different isolates may vary greatly in their content of pathogenicity factors. In fact, from both a general health and a vaccine perspective it may be equally critical to consider the immuno-modulatory pathogenicity mechanisms available within a person's microbiome along with specific bacterial species, as their combined effect may be very distinct or at least nonadditively amplified from individual factor contributions [76].

Another example of frequently observed chronic pathogen includes the highly prevalent immune distorting viruses of genus lymphocryptovirus comprising *Epstein-Barr virus* (*EBV*) and *Cytomegalovirus* (*CMV*); these cause lifelong infections, and *EBV* is known for its B-cell tropism. Both viruses can establish regulatory complex periods of latency. The effect of *EBV* and *CMV* infection versus age on immunity has recently been studied by Wang et al. where they differentiated age-dependent and -independent effects [77]. Specifically, decreased diversity of antibody repertoires with accumulation of memory B-cells and lower naive B-cell populations was associated with reduced vaccine efficacy in the elderly. They report that immune-globulin heavy chain (IGHV) mutation frequency increases upon infection with *CMV*, but not *EBV*. *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, where the effect may stem from the proportion of *CMV*-specific clones. Age and *EBV* infection correlated with persistent clonal expansion, where very few clonal lineages (possibly derived from a single ancestor) tend to be overrepresented. In the study these expanded clones may be cases of monoclonal B-cell lymphocytosis (MBL), a lymphoproliferative disorder with some characteristics of CLL typically seen in the elderly.

The inflammatory status including degree of immune system activation can have significant impact on vaccine efficacy. Recently it was shown in a YF-17D (yellow fever vaccine) trial comparing vaccination efficacy of 50 volunteers in Lausanne (Switzerland) versus the same number in Entebbe (Uganda) that the latter produced less effective humoral and $CD8^+$ responses. The authors negatively correlated the pre-existing activation level of $CD8^+$ T-cells and B-cells as well as pro-inflammatory monocytes at the time of vaccination with this reduced response [78]. Admittedly it

would also be interesting to know the cause of this inflammation, as the specific reason may affect the impact on vaccines. On the other hand the impact of pre-existing low-grade inflammatory conditions on vaccines is a recurring theme in the current review. In this context it is evident that determining protectivity profiles for vaccines is only one side of the coin. The other one is that the status of the vaccine recipient regarding inflammatory diseases, nutrition, and pre-existing immunity needs to be considered to understand inter-patient variability. Unfortunately the complex interaction of multiple clinical and subclinical infections is poorly understood. In the context of vaccines, inflammation and potential impact of chronically infecting pathogens and pathogen interactions need to be addressed.

5 Vaccines, Metabolism, Hormones, and the Nervous System

The interplay between metabolism and immune and nervous system is extensive and well beyond the scope of this review. However it is certainly beneficial to highlight some key concepts and current understanding regarding cross talk to show potential ramifications this may have on vaccine design. My aim here is to show factors similar to the preconditioning of chronically infecting pathogens impacting on the immunological environment within which a vaccine has to operate and which could be measured by a systems vaccinology approach. Indeed the relationship of inflammation and metabolic disorders has been extensively reviewed and is known to be very prominent [79]. Specifically the link and overlap between nutrient and pathogen-sensing mechanisms have been implicated in the development of inflammatory disorders. The biological rationale has been speculated to rest on the beneficial effect to coordinate short-time energy requirements during immune response with energy storage and metabolism, but the system has not evolved to deal with continuous nutrient surplus. Among others obesity, type 2 diabetes, cardiovascular disease, and certain neurologic disorders such as dementia and major depression have key low-grade (chronic) inflammatory components where this form of inflammation is distinct from acute inflammation involving swelling and pain. Low-grade inflammation is similar to classical inflammation on a molecular level, triggered by nutrients and metabolic surplus. It also turns out that immune components and metabolic organs may have evolved from the same source, as suggested by the fruit fly fat body which coordinates metabolic and pathogen-associated survival responses. Part of this link still seems to exist on pathway and physiological level. Examples are the lipopolysaccharide (LPS) receptor Toll-like receptor 4 (TLR4) which has been demonstrated 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 [80]. TLR4 polymorphisms also have been linked to likelihood of developing type II diabetes in the Chinese population [81]. GCN2 was identified by Querec et al. within a systems vaccinology analysis as a factor frequently contained in YF-17D vaccine response efficacy determinants. Several immune receptors, including TLR4, TLR2, and NOD1, have been shown to play a role in adipocyte inflammation [82]. TNF-alpha and other pro-inflammatory cytokines are over-expressed in adipose tissue and can lead to insulin resistance [83]. In fact adipocytes share a number of similarities with lymphocytes including pathogen-sensing capabilities. Also, lipids are well known for their capacity to regulate metabolism and adaptive and innate immunity, at least partially through peroxisome-proliferator-activated receptor (PPAR) and liver X receptor (LXR) family transcription factors, repressing expression of inflammatory mediators [79]. Interestingly drugs acting through PPARγ like thiazolidinediones are potent insulin sensitizers, but inhibit TLR-mediated activation of dendritic cells [84, 85]. It has been shown that catecholamines and adipokines influence immunity, metabolism, and the central nervous system [86]. Catecholamines including dopamine, noradrenaline, and adrenaline are generated by a number of cell types and can mediate a multitude of neural, metabolic, and pro- and anti-inflammatory effects. Adipose tissues as key endocrine organs secrete adipokines and hormones which serve a number of functions, including being both pro- and anti-inflammatory immune mediators with possibly a role in neuroinflammation [87–89]. Adipocytes can be found in a number of tissues and depending on location they can have different secretory profiles of important factors including 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 for prediction of vaccine response. While presence of local or systemic low-grade inflammatory markers may be more informative than obesity itself it has been shown to enhance susceptibility to infections and reduce immune competence and vaccine efficacy [90–96]. Recent murine studies also suggest a specific role of B-cells and autoantibodies in obesity-related pathology [97]. Type II diabetes is now understood to contain a significant inflammatory component and has been associated with reduced efficacy of hepatitis B vaccination in China [98–101]. Another study in this context suggests that although vaccine titers are reduced this is not necessarily associated with reduced protectivity, indicating that type II diabetes alone may not significantly reduce vaccine-provided protection, at least not in children [102].

Some of the factors that may influence the immune system during vaccination include age and degree of obesity. Among the other factors known to influence the immune system and vaccine response, hormones and specifically the group of progestogens,

hormone balance, and vitamins A and D are of particular relevance [103–105]. Hormones are considered to be the driver of immune differences between males and females, typically leading to weaker infection-related immune reactions in males and higher incidence of autoimmunity in females; intrinsically these observations are age dependent [106, 107]. Given current evidence, monitoring of hormone status should be a reasonable area for observation of future systems vaccinology studies and complements current procedures of monitoring serum proteins and other metabolites. Jensen et al. reviewed correlation of administered vaccines and vitamin A supplementation (VAS) including number of administrations. The data showed a significant sex-dependent difference (positive or negative) on mortality in monitored infants and VAS effect also depended on the location where it was administered (possibly because of ethnicities and associated genetic factors). Primarily, vitamin A supplementation is actively discussed as it is a WHO recommendation, and there is a differential effect on VAS-related vaccine responses between boys and girls [108, 109]. Independently it has also been reported that malnutrition overall has little effect on vaccine responses, suggesting that VAS administration at young age should be handled with care and sex dependency has to be further considered [110]. While oral but not parenteral vaccines are observed to be less effective in the developing world, the true impact of malnutrition, environmental enteropathy (EE), breast feeding, and coinfections is currently not well defined but a matter of ongoing clinical studies [111–116]. A currently proposed model is that altered condition of gut mucosa, microbiome, and metabolome negatively affects vaccine efficacy.

Protein CAMK4 (CaMKIV) may be part of the link between adaptive immunity, vaccines, and the nervous system. Fold change at day 3 post-vaccination with TIV is negatively correlated with antibody titers at day 28 via reduction of plasmablast expansion, and it is a well-known factor in T-cells and neuronal memory consolidation [12, 117]. The autonomous nervous system and the immune system cross-talk via the neuro-immune axis, misregulation of which is implicated in hypertension and cardiovascular disease [118]. Part of this regulation is that the brain can sense inflammatory cytokines and can modulate immune responses. The neurotransmitter acetylcholine can significantly attenuate the release of pro-inflammatory cytokines [119]. The gut as an organ of both central immunological and metabolic function is also a critical link to the microbiome and the gut-brain axis is implicated in the development of autoimmune and neurodevelopmental disorders [120]. In mice it has also been shown that defects in TLR5 lead to an altered microbiome and so-called metabolic syndrome (MeS) and transplantation of this altered microbiome to wild-type mice also confer features of MeS [121]. It is also becoming clear that there is a significant feedback between the endocrine system

and the microbiome, impacting on metabolism and immunity [122]. To further stress the role of the gut and signaling molecules aside of proteins in immune homeostasis, the role of bile acids as metabolic regulators with a part in inflammatory disorders and relationship to the gut microbiome has recently been reviewed [123]. In fact there is substantial evidence that psychological distress can predict gastrointestinal disorders and vice versa; animal studies suggest the role of early life conditioning in later health and disease. In this context it is important to better understand potential effects early life vaccination plans may have on shaping microbiota and hence this may lead to improved timing of vaccination schedules [124]. Major depressive disorders have been associated with the so-called metabolic syndrome and low-grade inflammation in the central nervous system including a role of adipokines leptin and ghrelin [125, 126]. The link between immunity and the nervous system is also of relevance because of the recurring assertions of vaccines contributing to certain neurological conditions. However, recently stronger vaccine responses against meningococcal conjugate vaccine in children with symptoms of depression and anxiety have been reported [127]. At this point it may be speculated whether the reported pro-inflammatory signature of carbohydrate-containing vaccines (MedImmune, Menactra) may be enhanced by a pre-existing low-grade inflammatory condition and which effect this would have on vaccines requiring different signatures such as TIV [2]. Another recent development is the potential recognition of narcolepsy as an autoimmune disorder. Narcolepsy has recently been associated with infectious diseases and specifically the 2009 *H1N1* (swine flu) pandemic and associated vaccine. This link does not seem to be definite, however, and may depend on genetic and ethnical background including the HLA-DQB1*06:02 genotype, additional unknown cofactors, and possibly vaccine formulation [128–131].

Taken together, metabolism and nervous and immune system are tightly interacting units. In fact, differentiating them may be more the result of an artificial concept than of a biological reality. Considering the known role of some 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 studies. Sampled tissues and data types will have to reflect the system a vaccine has to operate on. Specifically metabolomics, proteomics, and lipidomics should be valuable additions to currently pursued procedures in systems vaccinology. Yet it is currently unclear whether systemic determinants such as accessible in the blood-serum metabolome and lipidome may be sufficient to predict implications on vaccine responses, or whether local distribution is critical and well predicted by systemic concentration.

6 Glycans and Immunity

Recently the role of glycans in the immune system has been reviewed by Maverakis and colleagues, pointing out the various critical implications the glycocalyx of eukaryotic cells as well as glycosylations of serum proteins including antibodies have on modulating immunity [132]. While few data are available, analyzed in relationship to vaccines the implications for immunity have become clear, signaling the need to include the glycome in future systems vaccinology analysis. Indeed the glycome is heavily underrepresented in the majority of current systems biology investigations, which is arguably caused by experimental complexity. Yet current evidence shows that it is of critical importance in modulation of immunity and may provide numerous markers of use in understanding current and future reaction of individuals to vaccines. Also it should be considered that each antigen may be target of numerous antibodies where each may potentially be differently glycosylated, potentially grossly altering the effect from pro- 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 assumed to rest on anti-inflammatory antibodies, or specifically IgG with preference of anti-inflammatory Fc receptors [133]. The generation of anti-inflammatory glycosylation of IgG (specifically with terminal sialic acid) in tolerogenic therapies has recently been demonstrated to rest on antibody development in a non-inflammatory environment, suggesting the use of systems vaccinology for the deeper understanding of involved mechanisms and development of potentially supportive anti-inflammatory adjuvants [134]. Although the entire glycome can be analyzed using mass spectrometric approaches and great technical advances have recently been made using lectin microarrays and capillary electrophoresis, analysis of position-specific glycosylations is hindered by the non-template-based nature of glycosylations, as Maverakis et al. point out [135, 136]. Now we are in the unsatisfying situation to know there is a critical component to understanding immunity, but essentially lack tools equivalent in ease of use to other omics technologies. Optimal resolution of antibody class, isotype, and relative abundance of these should be part of any comprehensive analysis of antibody-mediated immune reactions. High titers do not necessarily mean desired effect if Fc regions of generated antibodies do not activate the intended lectins and/or Fc receptors [137, 138]. It is known that immunoglobulin Fc regions can take on hundreds of different structures with slightly or gravely different effects on targeted cell types and hence achieved effect in cancer, autoimmunity, and infectious diseases. Profiling of adjuvants should therefore consider the precise nature of produced antibodies, as biomedical effects can be

diverse and at least theoretically inverse to the intended. Changes in immunoglobulin glycosylation have been described for numerous autoimmune disorders and infectious diseases [139–141]. While there has to the author's knowledge been no definite proof that these changes are causative of disease they reflect changes in the immune system pinpointing towards biomarkers and very possibly at least contribute to development of an unbalanced immune phenotype. This assumption is based on the well-established dependence of immunoglobulin affinity to Fc receptors based on subclass and Fc glycosylation pattern as well as glycosylation of the receptor and resulting modification of antibody effect on various immune cell types [142, 143]. At least in humans pro- and anti-inflammatory effect of Fc gammaRII receptor isoforms (FcγRIIa and FcγRIIb, respectively) is well established [137]. In addition these receptors are differently responsive to single antibodies, where the majority is only responsive to immune complexes [132]. The overall effect of a particular antibody should therefore depend on affinity to Fc receptors (particularly pro- and anti-inflammatory) and relative abundance of these Fc receptors on specific target cells. Therefore unless an antibody interacts solely with FcγRIIb it may still also elicit pro-inflammatory signals.

In the case of the human pathogen dengue virus where antibody-dependent enhancement (ADE) is currently understood as a major driver of pathology, the role of FcγRIIA may primarily be enrichment of virus/antibody complexes on the cell surface [42]. However the second receptor variant FcγRIIb (that stimulates an anti-inflammatory effect) has been suggested to provide only limited ADE effect in spite of equivalent antibody Fc affinity, implicating that subtype of generated antibodies in dengue natural infection and likely also dengue vaccines may critically impact pathology and efficacy of the vaccines [144].

7 Conclusions

The last two decades have brought tremendous advancements in omics technology. Next-generation sequencing (NGS) but also microarray-based transcriptomics and peptide chips, proteomics, lipidomics, and glycomics have either been invented or significantly matured. Since the emergence of these technologies resulted in tremendous data volumes which need to be stored, analyzed, and brought into context the new science of system biology has emerged. The basic idea is to avoid reductionist approaches and view the entire investigated system and all measurable components in parallel to effectively observe system perturbations and cross talk. At the same time these advances have made immunology leap ahead, now allowing seeing many processes of health and disease in

the context of immunity. In many ways related arts and in this context especially vaccine development have sought to close the gap between basic research and application. While this is not always easy we now see the emergence of another fusion of sciences: systems vaccinology. Combining the methodologies of systems biology with the tools for interpretation stemming from modern immunology and focusing specifically at the improved understanding and development of vaccines this science has the potential to be a game changer for vaccine development. Many areas which have so far suffered from the immense complexity, interdependence and dynamic interplay of gene expression, interacting cell and tissue types, secretomes, metabolome, lipidome, genetic background, and immunological history present in immune systems are now offered an integrative approach to understand success and failure of vaccines and parameters to predict these early on. As such systems vaccinology has the intention to enhance understanding of vaccines, improve development processes of new vaccines, and possibly support clinical trials and personalized medicine. Specifically early identification of nonresponders and patient stratification in clinical trials may reduce the risk of future late-stage vaccine failures or should at least allow understanding of the reasons for failure. Also in already licensed vaccines systems vaccinology will allow deeper insights into determinants of efficacy including metabolic disorders especially those related to obesity, microbiome, and malnutrition. Ultimately it is a highly integrative development which will attempt to include as many views and technological options as possible, crossing barriers between disciplines. In fact modern immunology is already doing that, specifically when linking low-grade inflammation to metabolism and immune competence. This new understanding of disorders such as cardiovascular disease, type II diabetes and gastrointestinal dysfunction and their relationship with the microbiome is a major highlight of modern immunology. Therefore systems vaccinology only has to follow suit to bring modern vaccinology into a new era by integrating the impact of genes, environment, and the microbiome on protective immunity induced by vaccination.

References

1. Rappuoli R, Aderem A (2011) A 2020 vision for vaccines against HIV, tuberculosis and malaria. Nature 473:463–469
2. Pulendran B (2014) Systems vaccinology: probing humanity's diverse immune systems with vaccines. Proc Natl Acad Sci U S A 111:12300–12306
3. Querec TD, Akondy RS, Lee EK et al (2009) Systems biology approach predicts immunogenicity of the yellow fever vaccine in humans. Nat Immunol 10:116–125
4. Pulendran B (2009) Learning immunology from the yellow fever vaccine: innate immunity to systems vaccinology. Nat Rev Immunol 9:741–747
5. Pulendran B, Ahmed R (2011) Immunological mechanisms of vaccination. Nat Immunol 12:509–517
6. Pulendran B, Oh JZ, Nakaya HI et al (2013) Immunity to viruses: learning from successful human vaccines. Immunol Rev 255: |243–255

7. Barrett ADT, Teuwen DE (2009) Yellow fever vaccine - how does it work and why do rare cases of serious adverse events take place? Curr Opin Immunol 21:308–313
8. Biscayart C, Carrega MEP, Sagradini S et al (2014) Yellow fever vaccine-associated adverse events following extensive immunization in Argentina. Vaccine 32:1266–1272
9. Monath TP, Cetron MS, McCarthy K et al (2005) Yellow fever 17D vaccine safety and immunogenicity in the elderly. Hum Vaccin 1:207–214
10. Huang DW, Sherman BT, Tan Q et al (2007) DAVID bioinformatics resources: expanded annotation database and novel algorithms to better extract biology from large gene lists. Nucleic Acids Res 35:W169–W175
11. Aerts S, Thijs G, Coessens B et al (2003) Toucan: deciphering the cis-regulatory logic of coregulated genes. Nucleic Acids Res 31:1753–1764
12. Nakaya HI, Wrammert J, Lee EK et al (2011) Systems biology of vaccination for seasonal influenza in humans. Nat Immunol 12:786–795
13. Shen-Orr SS, Tibshirani R, Khatri P et al (2010) Cell type-specific gene expression differences in complex tissues. Nat Methods 7:287–289
14. Bucasas KL, Franco LM, Shaw CA et al (2011) Early patterns of gene expression correlate with the humoral immune response to influenza vaccination in humans. J Infect Dis 203:921–929
15. Vahey MT, Wang Z, Kester KE et al (2010) Expression of genes associated with immunoproteasome processing of major histocompatibility complex peptides is indicative of protection with adjuvanted RTS, S malaria vaccine. J Infect Dis 201:580–589
16. Furman D, Jojic V, Kidd B et al (2013) Apoptosis and other immune biomarkers predict influenza vaccine responsiveness. Mol Syst Biol 9:659
17. Furman D, Jojic V, Kidd B et al (2014) Apoptosis and other immune biomarkers predict influenza vaccine responsiveness. Mol Syst Biol 10:750
18. Tsang JS, Schwartzberg PL, Kotliarov Y et al (2014) Global analyses of human immune variation reveal baseline predictors of postvaccination responses. Cell 157:499–513
19. Locci M, Havenar-Daughton C, Landais E et al (2013) Human circulating PD-1+CXCR3-CXCR5+ memory Tfh cells are highly functional and correlate with broadly neutralizing HIV antibody responses. Immunity 39:758–769
20. Legutki JB, Johnston SA (2013) Immunosignatures can predict vaccine efficacy. Proc Natl Acad Sci USA 110:18614–18619
21. Tan PL, Jacobson RM, Poland GA et al (2001) Twin studies of immunogenicity – determining the genetic contribution to vaccine failure. Vaccine 19:2434–2439
22. Klein NP, Fireman B, Enright A et al (2007) A role for genetics in the immune response to the varicella vaccine. Pediatr Infect Dis J 26:300–305
23. Rubicz R, Leach CT, Kraig E et al (2011) Genetic factors influence serological measures of common infections. Hum Hered 72:133–141
24. Newport MJ, Goetghebuer T, Weiss HA et al (2004) Genetic regulation of immune responses to vaccines in early life. Genes Immun 5:122–129
25. Höhler T, Reuss E, Evers N et al (2002) Differential genetic determination of immune responsiveness to hepatitis B surface antigen and to hepatitis A virus: a vaccination study in twins. Lancet 360:991–995
26. Lee YC, Newport MJ, Goetghebuer T et al (2006) Influence of genetic and environmental factors on the immunogenicity of Hib vaccine in Gambian twins. Vaccine 24:5335–5340
27. Konradsen HB, Henrichsen J, Wachmann H, Holm N (1993) The influence of genetic factors on the immune response as judged by pneumococcal vaccination of mono- and dizygotic Caucasian twins. Clin Exp Immunol 92:532–536
28. O'Connor D, Pollard AJ (2013) Characterizing vaccine responses using host genomic and transcriptomic analysis. Clin Infect Dis 57:860–869
29. Ellis JA, Kemp AS, Ponsonby A-L (2014) Gene-environment interaction in autoimmune disease. Expert Rev Mol Med 16:e4
30. Franco LM, Bucasas KL, Wells JM et al (2013) Integrative genomic analysis of the human immune response to influenza vaccination. ELife 2:e00299
31. Lee EK (2007) Large-scale optimization-based classification models in medicine and biology. Ann Biomed Eng 35:1095–1109
32. Brooks JP, Lee EK (2008) Analysis of the consistency of a mixed integer programming-based multi-category constrained discriminant model. Ann Oper Res 174:147–168
33. Dabney AR (2005) Classification of microarrays to nearest centroids. Bioinformatics 21:4148–4154
34. Friedman J, Hastie T, Tibshirani R (2010) Regularization paths for generalized linear

models via coordinate descent. J Stat Softw 33:1–22
35. Li S, Rouphael N, Duraisingham S et al (2014) Molecular signatures of antibody responses derived from a systems biology study of five human vaccines. Nat Immunol 15:195–204
36. Obermoser G, Presnell S, Domico K et al (2013) Systems scale interactive exploration reveals quantitative and qualitative differences in response to influenza and pneumococcal vaccines. Immunity 38:831–844
37. Ngo ST, Steyn FJ, McCombe PA (2014) Gender differences in autoimmune disease. Front Neuroendocrinol 35:347–369
38. Beyer WE, de Bruijn IA, Palache AM et al (1999) Protection against influenza after annually repeated vaccination: a meta-analysis of serologic and field studies. Arch Intern Med 159:182–188
39. He X-S, Holmes TH, Sasaki S et al (2008) Baseline levels of influenza-specific CD4 memory T-cells affect T-cell responses to influenza vaccines. PLoS One 3:e2574
40. Sasaki S, He X-S, Holmes TH et al (2008) Influence of prior influenza vaccination on antibody and B-cell responses. PLoS One 3:e2975
41. Schmid MA, Diamond MS, Harris E (2014) Dendritic cells in dengue virus infection: targets of virus replication and mediators of immunity. Front Immunol 5:647
42. Chotiwan N, Roehrig JT, Schlesinger JJ et al (2014) Molecular determinants of dengue virus 2 envelope protein important for virus entry in FcγRIIA-mediated antibody-dependent enhancement of infection. Virology 456–457:238–246
43. Mustafa MS, Rasotgi V, Jain S, Gupta V (2015) Discovery of fifth serotype of dengue virus (DENV-5): a new public health dilemma in dengue control. Med J Armed Forces India 71:67–70
44. McElrath MJ, De Rosa SC, Moodie Z et al (2008) HIV-1 vaccine-induced immunity in the test-of-concept Step Study: a case-cohort analysis. Lancet 372:1894–1905
45. Perreau M, Pantaleo G, Kremer EJ (2008) Activation of a dendritic cell–T cell axis by Ad5 immune complexes creates an improved environment for replication of HIV in T cells. J Exp Med 205:2717–2725
46. Zak DE, Andersen-Nissen E, Peterson ER et al (2012) Merck Ad5/HIV induces broad innate immune activation that predicts CD8+ T-cell responses but is attenuated by preexisting Ad5 immunity. Proc Natl Acad Sci U S A 109:E3503–E3512
47. Nath I, Saini C, Valluri VL (2015) Immunology of leprosy and diagnostic challenges. Clin Dermatol 33:90–98
48. Lindquester GJ, Greer KA, Stewart JP, Sample JT (2014) Epstein-Barr virus IL-10 gene expression by a recombinant murine gammaherpesvirus in vivo enhances acute pathogenicity but does not affect latency or reactivation. Herpesviridae 5:1
49. Rawson TM, Anjum V, Hodgson J et al (2014) Leprosy and tuberculosis concomitant infection: a poorly understood, age-old relationship. Lepr Rev 85:288–295
50. Shankar EM, Velu V, Kamarulzaman A, Larsson M (2015) Mechanistic insights on immunosenescence and chronic immune activation in HIV-tuberculosis co-infection. World J Virol 4:17–24
51. Takem EN, Roca A, Cunnington A (2014) The association between malaria and non-typhoid Salmonella bacteraemia in children in sub-Saharan Africa: a literature review. Malar J 13:400
52. Coffey LL, Failloux A-B, Weaver SC (2014) Chikungunya virus-vector interactions. Viruses 6:4628–4663
53. Wu S, Jiang Z-Y, Sun Y-F et al (2013) Microbiota regulates the TLR7 signaling pathway against respiratory tract influenza A virus infection. Curr Microbiol 67:414–422
54. Oh JZ, Ravindran R, Chassaing B et al (2014) TLR5-mediated sensing of gut microbiota is necessary for antibody responses to seasonal influenza vaccination. Immunity 41:478–492
55. Wang J, Li F, Sun R et al (2013) Bacterial colonization dampens influenza-mediated acute lung injury via induction of M2 alveolar macrophages. Nat Commun 4:2106
56. Wang J, Li F, Wei H et al (2014) Respiratory influenza virus infection induces intestinal immune injury via microbiota-mediated Th17 cell-dependent inflammation. J Exp Med 211:2397–2410
57. Weber A, Zimmermann C, Kieseier BC et al (2014) Bacteria and their cell wall components uniformly co-activate interleukin-17-producing thymocytes. Clin Exp Immunol 178:504–515
58. Clarke TB (2014) Early innate immunity to bacterial infection in the lung is regulated systemically by the commensal microbiota via nod-like receptor ligands. Infect Immun 82:4596–4606
59. Kernbauer E, Ding Y, Cadwell K (2014) An enteric virus can replace the beneficial function of commensal bacteria. Nature 516: 94–98

60. Cadwell K (2014) Expanding the role of the virome: commensalism in the gut. J Virol 89:1951–1953
61. Li Q, Wang C, Tang C et al (2014) Dysbiosis of gut fungal microbiota is associated with mucosal inflammation in Crohn's disease. J Clin Gastroenterol 48:513–523
62. Leila Srour M, Marck KW, Baratti-Mayer D (2015) Noma: neglected, forgotten and a human rights issue. Int Health 7:149–150
63. Tilg H, Moschen AR (2015) Food, immunity, and the microbiome. Gastroenterology 148:1107–1119
64. Kamada N, Seo S-U, Chen GY, Núñez G (2013) Role of the gut microbiota in immunity and inflammatory disease. Nat Rev Immunol 13:321–335
65. Casanova J-L, Abel L (2013) The genetic theory of infectious diseases: a brief history and selected illustrations. Annu Rev Genomics Hum Genet 14:215–243
66. Le Chatelier E, Nielsen T, Qin J et al (2013) Richness of human gut microbiome correlates with metabolic markers. Nature 500:541–546
67. Ferreira RBR, Antunes LCM, Finlay BB (2010) Should the human microbiome be considered when developing vaccines? PLoS Pathog 6:e1001190
68. Kau AL, Ahern PP, Griffin NW et al (2011) Human nutrition, the gut microbiome and the immune system. Nature 474:327–336
69. Valdez Y, Brown EM, Finlay BB (2014) Influence of the microbiota on vaccine effectiveness. Trends Immunol 35:526–537
70. Huda MN, Lewis Z, Kalanetra KM et al (2014) Stool microbiota and vaccine responses of infants. Pediatrics 134:e362–e372
71. Lavelle A, Lennon G, O'Sullivan O et al (2015) Spatial variation of the colonic microbiota in patients with ulcerative colitis and control volunteers. Gut. doi:10.1136/gutjnl-2014-307873
72. Kurata S, Osaki T, Yonezawa H et al (2014) Role IL-17A and IL-10 in the antigen induced inflammation model by Mycoplasma pneumoniae. BMC Microbiol 14:156
73. Spuesens EBM, Fraaij PLA, Visser EG et al (2013) Carriage of Mycoplasma pneumoniae in the upper respiratory tract of symptomatic and asymptomatic children: an observational study. PLoS Med 10:e1001444
74. Ben Aissa-Fennira F, Sassi A, Bouguerra A, Benammar-Elgaaied A (2011) Immunoregulatory role for a public IgM idiotype in the induction of autoimmune diseases in Mycoplasma pneumoniae infection. Immunol Lett 136:130–137
75. Stipkovits L, Egyed L, Palfi V et al (2012) Effect of low-pathogenicity influenza virus H3N8 infection on Mycoplasma gallisepticum infection of chickens. Avian Pathol 41:51–57
76. Xiao L, Crabb DM, Dai Y et al (2014) Suppression of antimicrobial peptide expression by ureaplasma species. Infect Immun 82:1657–1665
77. Wang C, Liu Y, Xu LT et al (2014) Effects of aging, cytomegalovirus infection, and EBV infection on human B cell repertoires. J Immunol 192:603–611
78. Muyanja E, Ssemaganda A, Ngauv P et al (2014) Immune activation alters cellular and humoral responses to yellow fever 17D vaccine. J Clin Invest 124:3147–3158
79. Hotamisligil GS (2006) Inflammation and metabolic disorders. Nature 444:860–867
80. Ravindran R, Khan N, Nakaya HI et al (2014) Vaccine activation of the nutrient sensor GCN2 in dendritic cells enhances antigen presentation. Science 343:313–317
81. Peng D, Jiang F, Zhang R et al (2014) Association of Toll-like Receptor 4 Gene polymorphisms with susceptibility to type 2 diabetes mellitus in the Chinese population. J Diabetes 7:485–492
82. Spurohit J, Hu P, Burke SJ et al (2013) The effects of NOD activation on adipocyte differentiation. Obesity (Silver Spring) 21:737–747
83. Krogh-Madsen R, Plomgaard P, Møller K et al (2006) Influence of TNF-α and IL-6 infusions on insulin sensitivity and expression of IL-18 in humans. Am J Physiol Endocrinol Metab 291:E108–E114
84. Appel S, Mirakaj V, Bringmann A et al (2005) PPAR-gamma agonists inhibit toll-like receptor-mediated activation of dendritic cells via the MAP kinase and NF-kappaB pathways. Blood 106:3888–3894
85. Ahmadian M, Suh JM, Hah N et al (2013) PPARγ signaling and metabolism: the good, the bad and the future. Nat Med 19:557–566
86. Barnes MA, Carson MJ, Nair MG (2015) Non-traditional cytokines: how catecholamines and adipokines influence macrophages in immunity, metabolism and the central nervous system. Cytokine 72:210–219
87. Aguilar-Valles A, Inoue W, Rummel C, Luheshi GN (2015) Obesity, adipokines and neuroinflammation. Neuropharmacology 96(PtA):124–134
88. Stojsavljević S, Gomerčić Palčić M, Virović Jukić L et al (2014) Adipokines and proin-

flammatory cytokines, the key mediators in the pathogenesis of nonalcoholic fatty liver disease. World J Gastroenterol 20: 18070–18091
89. Ouchi N, Parker JL, Lugus JJ, Walsh K (2011) Adipokines in inflammation and metabolic disease. Nat Rev Immunol 11:85–97
90. Chen S, Akbar SMF, Miyake T et al (2015) Diminished immune response to vaccinations in obesity: Role of myeloid-derived suppressor and other myeloid cells. Obes Res Clin Pract 9:35–44
91. Young KM, Gray CM, Bekker L-G (2013) Is obesity a risk factor for vaccine non-responsiveness? PLoS One 8:e82779
92. Park H-L, Shim S-H, Lee E-Y et al (2014) Obesity-induced chronic inflammation is associated with the reduced efficacy of influenza vaccine. Hum Vaccin Immunother 10:1181–1186
93. Lumeng CN, Saltiel AR (2011) Inflammatory links between obesity and metabolic disease. J Clin Invest 121:2111–2117
94. Lamas O, Marti A, Martínez JA (2002) Obesity and immunocompetence. Eur J Clin Nutr 56(Suppl 3):S42–S45
95. Genoni G, Prodam F, Marolda A et al (2014) Obesity and infection: two sides of one coin. Eur J Pediatr 173:25–32
96. Prathibha Bandaru HR, Nappanveettil G (2013) The impact of obesity on immune response to infection and vaccine: an insight into plausible mechanisms. Endocrinol Metab Synd 2:113
97. Shaikh SR, Haas KM, Beck MA, Teague H (2015) The effects of diet-induced obesity on B cell function. Clin Exp Immunol 179:90–99
98. Donath MY, Shoelson SE (2011) Type 2 diabetes as an inflammatory disease. Nat Rev Immunol 11:98–107
99. Donath MY (2014) Targeting inflammation in the treatment of type 2 diabetes: time to start. Nat Rev Drug Discov 13:465–476
100. Dhillon S, Moore C, Li SD et al (2012) Efficacy of high-dose intra-dermal hepatitis B virus vaccine in previous vaccination non-responders with chronic liver disease. Dig Dis Sci 57:215–220
101. Li W, Wei Z, Cai L et al (2011) Effect of type 2 diabetes mellitus on efficacy of hepatitis B vaccine and revaccination strategy. Med J Chin Peoples Lib Army 36:1068–1070
102. Leonardi S, Vitaliti G, Garozzo MT et al (2012) Hepatitis B vaccination failure in children with diabetes mellitus? The debate continues. Hum Vaccin Immunother 8:448–452
103. Tan IJ, Peeva E, Zandman-Goddard G (2015) Hormonal modulation of the immune system - a spotlight on the role of progestogens. Autoimmun Rev. doi:10.1016/j.autrev.2015.02.004
104. Pettengill MA, van Haren SD, Levy O (2014) Soluble mediators regulating immunity in early life. Front Immunol 5:457
105. Jensen KJ, Ndure J, Plebanski M, Flanagan KL (2015) Heterologous and sex differential effects of administering vitamin A supplementation with vaccines. Trans R Soc Trop Med Hyg 109:36–45
106. Furman D (2015) Sexual dimorphism in immunity: improving our understanding of vaccine immune responses in men. Expert Rev Vaccines 14:461–471
107. Gubbels Bupp MR (2015) Sex, the aging immune system, and chronic disease. Cell Immunol 294(2):102–110
108. Ahmad SM, Raqib R, Qadri F, Stephensen CB (2014) The effect of newborn vitamin A supplementation on infant immune functions: trial design, interventions, and baseline data. Contemp Clin Trials 39:269–279
109. Fisker AB, Bale C, Rodrigues A et al (2014) High-dose vitamin A with vaccination after 6 months of age: a randomized trial. Pediatrics 134:e739–e748
110. Savy M, Edmond K, Fine PEM et al (2009) Landscape analysis of interactions between nutrition and vaccine responses in children. J Nutr 139:2154S–2218S
111. Patriarca PA, Wright PF, John TJ (1991) Factors affecting the immunogenicity of oral poliovirus vaccine in developing countries: review. Rev Infect Dis 13:926–939
112. Kirkpatrick BD, Colgate ER, Mychaleckyj JC et al (2015) The "Performance of Rotavirus and Oral Polio Vaccines in Developing Countries" (PROVIDE) study: description of methods of an interventional study designed to explore complex biologic problems. Am J Trop Med Hyg 92:744–751
113. Hoest C, Seidman JC, Pan W et al (2014) Evaluating associations between vaccine response and malnutrition, gut function, and enteric infections in the MAL-ED cohort study: methods and challenges. Clin Infect Dis 59(Suppl 4):S273–S279
114. MAL-ED Network Investigators (2014) The MAL-ED study: a multinational and multidisciplinary approach to understand the relationship between enteric pathogens, malnutrition, gut physiology, physical growth, cognitive development, and immune responses in infants and children up to 2 years of age in

resource-poor environments. Clin Infect Dis 59(Suppl 4):S193–S206
115. Haque R, Snider C, Liu Y et al (2014) Oral polio vaccine response in breast fed infants with malnutrition and diarrhea. Vaccine 32:478–482
116. Qadri F, Bhuiyan TR, Sack DA, Svennerholm A-M (2013) Immune responses and protection in children in developing countries induced by oral vaccines. Vaccine 31:452–460
117. Fukushima H, Maeda R, Suzuki R et al (2008) Upregulation of calcium/calmodulin-dependent protein kinase IV improves memory formation and rescues memory loss with aging. J Neurosci Off J Soc Neurosci 28:9910–9919
118. Abboud FM, Harwani SC, Chapleau MW (2012) Autonomic neural regulation of the immune system: implications for hypertension and cardiovascular disease. Hypertension 59:755–762
119. Borovikova LV, Ivanova S, Zhang M et al (2000) Vagus nerve stimulation attenuates the systemic inflammatory response to endotoxin. Nature 405:458–462
120. Sherman MP, Zaghouani H, Niklas V (2015) Gut microbiota, the immune system, and diet influence the neonatal gut-brain axis. Pediatr Res 77:127–135
121. Vijay-Kumar M, Aitken JD, Carvalho FA et al (2010) Metabolic syndrome and altered gut microbiota in mice lacking Toll-like receptor 5. Science 328:228–231
122. Neuman H, Debelius JW, Knight R, Koren O (2015) Microbial endocrinology: the interplay between the microbiota and the endocrine system. FEMS Microbiol Rev 39:509–521
123. Li T, Chiang JYL (2015) Bile acids as metabolic regulators. Curr Opin Gastroenterol 31:159–165
124. Keightley PC, Koloski NA, Talley NJ (2015) Pathways in gut-brain communication: Evidence for distinct gut-to-brain and brain-to-gut syndromes. Aust N Z J Psychiatry 49:207–214
125. Marazziti D, Rutigliano G, Baroni S et al (2014) Metabolic syndrome and major depression. CNS Spectr 19:293–304
126. Bakunina N, Pariante CM, Zunszain PA (2015) Immune mechanisms linked to depression via oxidative stress and neuroprogression. Immunology 144:365–373
127. O'Connor TG, Moynihan JA, Wyman PA et al (2014) Depressive symptoms and immune response to meningococcal conjugate vaccine in early adolescence. Dev Psychopathol 26:1567–1576
128. Arango M-T, Kivity S, Chapman J, Shoenfeld Y (2014) Narcolepsy – genes, infections and vaccines: the clues for a new autoimmune disease. Isr Med Assoc J 16:636–637
129. Duffy J, Weintraub E, Vellozzi C et al (2014) Narcolepsy and influenza A(H1N1) pandemic 2009 vaccination in the United States. Neurology 83:1823–1830
130. Vaarala O, Vuorela A, Partinen M et al (2014) Antigenic differences between AS03 adjuvanted influenza A (H1N1) pandemic vaccines: implications for pandemrix-associated narcolepsy risk. PLoS ONE 9, e114361
131. Partinen M, Kornum BR, Plazzi G et al (2014) Narcolepsy as an autoimmune disease: the role of H1N1 infection and vaccination. Lancet Neurol 13:600–613
132. Maverakis E, Kim K, Shimoda M et al (2015) Glycans in the immune system and The Altered Glycan Theory of Autoimmunity: a critical review. J Autoimmun. doi:10.1016/j.jaut.2014.12.002
133. Anthony RM, Nimmerjahn F (2011) The role of differential IgG glycosylation in the interaction of antibodies with FcγRs in vivo. Curr Opin Organ Transplant 16:7–14
134. Oefner CM, Winkler A, Hess C et al (2012) Tolerance induction with T cell-dependent protein antigens induces regulatory sialylated IgGs. J Allergy Clin Immunol 129:1647–1655, e13
135. Hirabayashi J, Yamada M, Kuno A, Tateno H (2013) Lectin microarrays: concept, principle and applications. Chem Soc Rev 42:4443–4458
136. Mahan AE, Tedesco J, Dionne K et al (2015) A method for high-throughput, sensitive analysis of IgG Fc and Fab glycosylation by capillary electrophoresis. J Immunol Methods 417:34–44
137. Pincetic A, Bournazos S, DiLillo DJ et al (2014) Type I and type II Fc receptors regulate innate and adaptive immunity. Nat Immunol 15:707–716
138. Collin M, Ehlers M (2013) The carbohydrate switch between pathogenic and immunosuppressive antigen-specific antibodies. Exp Dermatol 22:511–514
139. Selman MHJ, Niks EH, Titulaer MJ et al (2011) IgG fc N-glycosylation changes in Lambert-Eaton myasthenic syndrome and myasthenia gravis. J Proteome Res 10:143–152
140. Goulabchand R, Vincent T, Batteux F et al (2014) Impact of autoantibody glycosylation in autoimmune diseases. Autoimmun Rev 13:742–750

141. Gardinassi LG, Dotz V, Hipgrave Ederveen A et al. (2014) Clinical severity of visceral leishmaniasis is associated with changes in immunoglobulin g fc N-glycosylation. mBio 5:e01844.
142. Nimmerjahn F, Ravetch JV (2005) Divergent immunoglobulin g subclass activity through selective Fc receptor binding. Science 310:1510–1512
143. Hayes JM, Frostell A, Cosgrave EFJ et al (2014) Fc gamma receptor glycosylation modulates the binding of IgG glycoforms: a requirement for stable antibody interactions. J Proteome Res 13:5471–5485
144. Boonnak K, Slike BM, Donofrio GC, Marovich MA (2013) Human FcγRII cytoplasmic domains differentially influence antibody-mediated dengue virus infection. J Immunol 190:5659–5665