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Immunogenomics and systems biology of vaccines

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Summary

Vaccines represent a potent tool to prevent or contain infectious diseases with high morbidity or mortality. However, despite their widespread use, we still have a limited understanding of the mechanisms underlying the effective elicitation of protective immune responses by vaccines. Recent research suggests that this represents the cooperative action of the innate and adaptive immune systems. Immunity is made of a multifaceted set of integrated responses involving a dynamic interaction of thousands of molecules, whose list is constantly updated to fill the several empty spaces of this puzzle. The recent development of new technologies and computational tools permits the comprehensive and quantitative analysis of the interactions between all of the components of immunity over time. Here, we review the role of the innate immunity in the host response to vaccine antigens and the potential of systems biology in providing relevant and novel insights in the mechanisms of action of vaccines to improve their design and effectiveness.

Keywords

innate immunity; PRRs; PAMPs; TLRs; APCs; adaptive immunity

Vaccine development

An effective vaccine needs to mimic as close as possible the ‘real’ biological entity from which it is derived (i.e. pathogen, cancer cell) in order to be recognized by the host immune system as real ‘danger’. This will eventually initiate the cascade of molecular and cellular events inducing several levels of cross-talk between the innate and adaptive immune systems for an effective immune response and immunological memory. However, most successful vaccines have been empirically derived, and the immunological mechanisms underlying the effective induction of long-term protective immunity remain largely unknown (1, 2).

Most of the current successful vaccines are based on live attenuated or inactivated pathogen ‘particles’ carrying their own unique and specific antigens. The live attenuated vaccines are characterized by a limited viral replication in the host upon injection and carry the native pathogen-associated molecular signals (PAMS) (i.e. viral genetic material), which trigger the activation of the innate immune system binding to the pathogen recognition receptors (PRRs). Live attenuated vaccines replicate and reach host immune sites where they are taken up by dendritic cells (DCs) or other antigen-presenting cells (APCs), which migrate to

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lymphoid organs for presenting the antigens to T and B lymphocytes. They elicit immune responses similar to those from natural infections and often are effective after a single administration (3). However, such vaccines may cause mild-to-severe adverse effects in patients, often as consequence of the limited replication in the host.

On the contrary, the inactivated vaccines do not replicate and are safer than live attenuated vaccines; however, they are generally less effective, requiring multiple administrations to boost the immune response antibody titer over time. The inactivated vaccines are made as whole cell or as subunit vaccines (i.e. individual viral proteins). In this framework, recent advances in genomics and proteomics have provided essential tools to develop alternative non-replicating vaccine strategy, including recombinant proteins, synthetic peptides, DNA, and particulate structures (i.e. virus-like particles).

Inactivated as well as non-replicating vaccines activate innate responses only at their site of injection, and intradermal skin immunization seems to induce a more effective protective immune responses (4–6), considering the high number of DCs in the skin dermis (7). In contrast to safety advantages, the major drawback of vaccines based on selected antigens is the less effective processing and presentation to the immune system. Therefore, most formulations of non-living vaccines must include an adjuvant as ‘danger’ signal to trigger a sufficient activation of the innate system and, downstream, of the adaptive immune response (8).

Innate immunity and vaccine recognition

Research over the past decade has revealed a fundamental role for the innate immune system in sensing microbes or viruses and regulating the strength and quality of the adaptive immune responses to that microbe (9, 10). The innate immune system consists of several interacting cell types, including DCs, macrophages, epithelial cells, endothelial cells, natural killer (NK) cells, NK T cells, basophils, and mast cells, which are involved in ‘sensing’ microbes or viruses and initiating immunity against them. DCs plays a key role in directly sensing the presence of pathogens and orchestrating the interactions between the other innate immune cell types and facilitating the elicitation of anti-viral defenses, such secretion of type I interferons (IFNs) and defensins (11, 12). In addition to their roles in sensing pathogens and orchestrating innate immune defenses, DCs also play a critical role in translating innate immunity into adaptive immunity (13, 14). Understanding the impact of innate immunity on the regulation of adaptive immunity, and harnessing such knowledge to induce optimal immunity to human immunodeficiency virus (HIV), was recognized as an area of the highest importance.

The innate immune system is able to sense microbial or viral stimuli by the expression of so-called PRRs, which are expressed constitutively in the host on cells of the innate immune system, such as DCs (9, 10, 14–16), activating specific signaling pathways to drive biological and immunological responses. Among the PRRs, a key role is played by Toll-like receptors (TLRs), which are widely expressed on innate immune cells (including DCs, macrophages, mast cells, neutrophils), endothelial cells, and fibroblasts (9, 10, 16–20).

TLRs are a family of 12 type I integral membrane glycoproteins with extracellular domains containing varying numbers of leucine-rich-repeat motifs and a cytoplasmic signaling domain homologous to that of the interleukin 1 receptor (IL-1R), termed the Toll/IL-1R homology (TIR) domain (21). TLRs 1, 2, 4, 5, and 6 are expressed on the cell surface, whereas TLR3, 7, 8, and 9 are found almost exclusively in intracellular compartments such as endosomes (9, 10, 14–16).

TLRs recognize structural components shared by many bacteria, viruses, and fungi (17). Examples of such components include lipopolysaccharides (LPSs) (recognized by TLR4) (22, 23), lipopeptides (by cooperation of TLR2 with TLR1 or TLR6) (24–27), viral single- or double-stranded RNA (by TLR7 with TLR8 and by TLR3, respectively) (28–31), bacterial or viral DNA containing CpG motifs (by TLR9) (32, 33), and flagellin (by TLR5) (34).

In addition to TLRs, other families of PRRs such as the C-type lectins and nuclear-binding oligomerization domain (NOD) proteins are involved in sensing microbial stimuli and modulating immune responses (15, 35–37). The C-type lectins such as DC-specific inter-cellular adhesion molecule-3 (ICAM-3) grabbing non-integrin (DC-SIGN) and DC-associated C-type lectin-1 (Dectin-1) recognize molecules of pathogens such as HIV, hepatitis C virus (HCV), *Helicobacter pylori*, and *Mycobacterium tuberculosis* (38). NOD proteins recognize components of intracellular bacteria (36). Furthermore, intracellular RNA helicases RIG-I and melanoma differentiation-associated gene 5 (also called helicard) can sense dsRNA (39–41).

The interaction between PRRs and components of microbes or viruses triggers a downstream signaling cascade leading to several cellular processes, including production of proinflammatory cytokines and chemokines (42). Signaling intermediates of TLR activation include myeloid differentiation factor-88 (MyD88), TIR-associated-protein (TIRAP), also known as MAL, Toll receptor-associated activator of interferon, Toll receptor-associated molecule, IL-1 receptor-associated kinases (IRAK), and tumor necrosis factor (TNF) receptor-associated factor 6 (15, 42). The endpoint of this signaling cascade is the activation of transcription factors [IFN regulatory factor (IRF)3, IRF7, AP-1, NF-κB] inducing the activation of inflammatory cytokine genes, such as TNF-β, IL-6, IL-1β, and IL-12, as well as the upregulation of costimulatory molecules such as CD80, CD86, CD40 on DCs.

Many of the best empirically derived vaccines and adjuvants mediate their efficacy by activating specific innate immune receptors. For example, the highly effective yellow fever vaccine-17D, one of the most successful vaccines that has been administered to over half a billion people globally, signals via at least four different TLRs as well as RIG-I like receptors to elicit a broad spectrum of T-cell responses (43, 44). This suggests that the immune response generated by a live attenuated vaccine can be effectively mimicked by adjuvants composed of the appropriate TLR and/or non-TLR ligands. Consistent with this, it was recently shown that the superior immunogenicity of the inactivated whole virus H5N1 influenza vaccine is primarily controlled by TLR signaling (45), and the *Haemophilus influenzae* type b-outer membrane protein complex glycoconjugate vaccine induces cytokine production by engaging human TLR2 and requires the presence of TLR2 for optimal immunogenicity (46). In addition, the Bacillus Calmette–Guerin (BCG) has been shown to engage TLR2 and TLR4 (47, 48), although the consequence of this engagement for adaptive immunity is not known. Furthermore, recent work suggests that some adjuvants can induce robust adaptive immunity in a TLR-independent manner, perhaps through other receptors in the innate immune system (49). For example, it was recently demonstrated that alum, the only adjuvant that was licensed for several decades, signals through the NALP3 inflammasome (50–52), as DCs or macrophages stimulated *in vitro* with alum plus LPS induce IL-1β and IL-18 in a manner dependent on caspase-1 and NALP3 (50–52). However, whether NALP3 is required for the adjuvanticity of alum remains controversial, with some studies demonstrating abrogation of antibody responses in *Nalp3*$^{-/-}$ mice and other studies showing partial or no effects.

Exploiting vaccine recognition by PRRs

For most currently licensed vaccines, the degree of engagement of TLRs has not been studied, with a few exceptions. In particular, the live attenuated yellow fever vaccine has been demonstrated to activate multiple DC subsets via TLRs 2, 7, 8, and 9 (43). For many other licensed vaccines, the engagement of TLRs has not been documented, but it is possible to guess based on studies performed on the original virus from which the vaccine is derived. Single-stranded RNA of live attenuated (cold adapted) influenza vaccines, indeed, are likely to activate TLR3 and TLR7 during intracellular replication, leading to the upregulation of inflammatory cytokines (53, 54). Similarly, bacterial and viral DNA containing unmethylated CpG motifs activate TLR9 (55, 56).

There is little or no evidence that the immunogenicity of vaccines composed of killed pathogens or subunit elements is mediated by TLR engagement, possibly explaining the generally observed lower and short-lasting immune response. Adjuvanting formulation, in these cases, may significantly improve vaccine effectiveness. However, the number of adjuvants approved for human use is quite limited, including alum, MF59 (57), monophosphoryl lipid A (MPL) (58), AS04 (consisting of MPL adsorbed on alum) (59, 60), immunopotentiating reconstituted influenza virosomes (61, 62), and cholera toxin B subunit (63). Of these, only MPL is known to engage a TLR (TLR4), being a non-toxic derivative of the LPS of *Salmonella minnesota*. Consequently, many new TLR-specific vaccine adjuvants are under development and are being evaluated in preclinical and human clinical trials (64–68).

TLR signaling for potent and prolonged adaptive immune responses

Adaptive immune responses are initiated in the T-cell-rich areas of lymph nodes, where naive T cells undergo to clonal expansion and differentiation into effector cells upon activation mediated by migrating antigen-bearing DCs. In particular, antigen-specific activated $CD4^+$ T-helper (Th) cells can be directed into a Th1, Th2, or T-regulatory (Treg) polarization upon direct contact with antigens and induction by specific cytokines (69–74). Consequently, the T-helper cells' polarization will ultimately lead the adaptive immune system toward either a cellular T cell, sustained by $CD8^+$ cytotoxic T lymphocytes (Th1), or a humoral antibody (Th2), or a tolerance (Treg) response.

To establish immunological memory able to promptly respond to subsequent encounters with the same antigen, a subset of polarized activated effector T cells will differentiate into long-lasting memory T cells (75), which is the ultimate goal of vaccination strategies. Although most successful vaccines in human medicine induce a long-lasting protective humoral adaptive immune response (76–78), antibodies seem to only partially contribute to protective immune response in other vaccines (79, 80). In the last years, indeed, the development of vaccines able to elicit an effective cellular adaptive immune response is considered of high priority for chronic infections, such as HCV and HIV infections (81–84) or cancer (85–87).

In this respect, the engagement of TLRs by the vaccine antigen would be extremely beneficial, resulting mainly in the production of Th-1 cytokines (88). The TLR3 ligand polyI:C, a synthetic analog of dsRNA, has been shown to have a potent adjuvanting effect in several experimental systems (89–91). TLR7/8 agonists have been shown to improve the magnitude and quality of memory T-cell responses elicited by HIV Gag protein (92). The TLR9 agonist bacterial unmethylated CpG DNA has shown a significant therapeutic potential in cancer, infectious disease, and asthma (93–97).

Besides having the capacity of inducing a Th1-type immune response, TLR ligands have been shown to amplify Th2 responses (98–101). In particular, bacterial lipoproteins exert adjuvant function in Lyme disease vaccination through TLR1 (24, 102), and flagellin has been shown to enhance immune response to influenza vaccine through TLR5 (103). Furthermore, polyI:C and CpG DNA improve the survival of activated $CD4^+$ Th cells even in the absence of APCs (104) as well as generate fully functional $CD8^+$ memory cells without any $CD4^+$ T-cell help (105, 106). Furthermore, TLR7/8 agonist has been shown to enhance both effector and memory T-cell responses to HIV gag antigen (92).

Systems biology in vaccine studies

The innate immune system is at the interface between the vaccine antigen and the host's adaptive immune response; therefore, the evaluation of the molecular effects induced by vaccines on PRRs biology is of high relevance. Studying molecular signatures that are induced rapidly after vaccination will identify causal elements of the adaptive immune response, which may be useful in ultimately predicting protective immune responses. Such prediction will enable the evaluation of the efficacy or immunogenicity of untested vaccines in the general population or the identification of unresponsive individuals to vaccination. Furthermore, the predictive signatures would uncover new correlates of protection and further decipher the biological mechanisms by which such molecular signatures modulate vaccine-induced immunity and protection.

Systems biology approaches provide detailed level of investigation to better and fully analyze the network of interactions within the *ménage à trois* of this game (vaccine, innate, and adaptive immunity). Conversely to traditional 'reductionist' approach, the paradigm of systems biology is to look at a biological system as a whole, evaluating interactions among biological elements and their relationship with the surrounding environment. Systems biology has been increasingly applied to oncology (107–109), autoimmunity and infections (110, 111), and only recently to vaccinology (Fig. 1).

Transcriptomics, proteomics, and genetics

Transcriptomics applied to the immune response enables the identification of specific set of genes and pathways differentially regulated upon encounter with a foreign antigen, and several new insights into interactions between pathogens and innate immunity have been identified (112–117). However, to reduce the number of meaningless observations, results generated by transcriptomics studies need to be integrated by meta-analysis performed on multiple independent datasets, which requires access to several datasets. Several publicly available databases of immunology-related transcriptomic datasets have been created in the recent years (118–121). Furthermore, to improve integration of immunology datasets of these different databases, the Immunological Genome Project initiative has been established recently with the ambitious goal to combine immunology and computational biology laboratories in a systems-level approach (122).

The possibility of performing meta-analysis has enabled the identification of an expression signature, designated as the 'common host response', characterized by a cluster of 511 genes selectively and consistently induced in several cell types upon exposure to different pathogens (114). The described expression signature includes cytokine genes, IFN-stimulated genes, transcriptional factors and components of signal transduction pathways, genes that limit the pathogenetic consequences of the immune response, and, more interestingly, genes that have not previously been associated with the immune response. The identification of this shared transcriptional program to foreign antigens among different host cells, even outside of the immune system, suggests the evolution of a multi-cellular and multi-compartmental line of host defense to infection.

Proteomics gives a comprehensive picture of the immune interactome, representing the interactions involving the host and pathogen genes and gene products known to participate in the immune response. Several viral proteins, in particular of HCV, have been identified to interact with key proteins in innate immunity pathways and are involved in the viral immune evasion (123–125).

In this respect, databases on interactions between proteins of innate immunity and pathogens have been developed in the last few years, representing invaluable tools for studying the immune interactome (125–129). In particular, the Innate DB includes up to 7000 innate immunity-relevant interactions involving 2000 human and mouse genes (130).

In addition to studies on protein-to-protein interactions, the so-called 'phosphoproteomics' is focused on the dynamics of the innate immune response protein phosphorylation signaling cascades, to identify the complete 'kinome' of a cell (131, 132). A further approach is represented by large-scale RNA interference screens, where genes are progressively knocked down using appropriate inhibitory RNA molecules, which have enabled the identification of host proteins involved in HIV and West Nile virus infections (133, 134). Similarly, the profiling of microRNA (miRNA) expression can provide valuable information about the role of these posttranscriptional regulators of gene expression on the modulation of genes relevant to innate immunity and, therefore, the immune response itself, as recently shown for the leukocyte miRNA response to LPS (135–137).

The study of genetic polymorphisms represents an additional level of analysis for the global evaluation of factors involved in the host response to foreign antigens. Polymorphisms can adversely affect expression of genes as well as proteins of the innate immune system and, consequently, host-pathogen interactions as well as molecular signaling (138–141). Implications of polymorphisms in TLR genes on infectious diseases progression have been reported (142–150). In particular, the role of polymorphisms in TLR9 gene and clinical course of HIV-1 infection (151) as well as susceptibility to tuberculosis and specific polymorphisms in the TLR2 gene have been described (152–154). These findings are controversial and not consistently confirmed (155–157).

Multiparametric analyses for prediction of vaccine immunogenicity

Systems level studies have been recently performed to identify gene 'signatures' in humans predicting immune responses to yellow fever vaccine (YF-17D) in humans (158, 159). Upon vaccination with YF-17D, a variable response over time is observed, in terms of both magnitude of the antigen-specific $CD8^+$ T-cell responses and neutralizing antibody titers. In parallel, gene transcriptional profile in PBMCs from vaccinated individuals showed the induction of a molecular signature, including several immune genes involved in innate sensing of viruses and antiviral immunity, which lasted for more than 2 weeks post vaccination (158, 159). Among these, genes were identified encoding innate sensing receptors (i.e. TLR7, RIG-I), transcription factors that regulate the expression of type I IFNs, IRF7, and signal transducer and activator of transcription 1 (STAT1). Furthermore, genes encoding proteins in the complement pathway (i.e. C1QB) and the inflammasome were induced. In particular, a group of transcription factors were identified as key regulators of the early innate immune response to the YF-17D vaccine (158, 159), such as ETS2, whose expression is upregulated in activated and proliferating T cells (160, 161) and induces IL-12 p40 (Th1) and IL-5 (Th2) gene expression (162, 163). The enhanced transcription of downstream genes involved in the maturation and differentiation of T cells, B cells, NK cells, and macrophages was observed (159).

There was no significant correlation, however, between the induction of these genes and the magnitude of the $CD8^+$ T-cell or neutralizing antibody response, suggesting that the

observed molecular signature might be consequent to the replication of the vaccine live attenuated virus. However, two genes – solute carrier family 2, member 6 (SLC2A6), and eukaryotic translation initiation factor 2 α kinase 4 (EIF2AK4) – were found to strongly correlate with the magnitude of antigen-specific $CD8^+$ T-cell responses and antibody titers in a second YF-17D vaccine trial (158).

EIF2AK4 regulates protein synthesis in response to environmental stresses by phosphorylating elongation initiation factor 2α (eIF2α) (164, 165). Indeed, YF-17D vaccination induced the phosphorylation of eIF2α as well as the formation of stress granules, and other genes involved in the stress response pathway correlated with the $CD8^+$ T-cell response (158). Moreover, the TNF receptor superfamily, receptor 17 (TNFRSF17), which is a receptor for B-cell-activating factor belonging to the TNF family (BAFF), predicted the neutralizing antibody response with highly significant accuracy (158). Finally, PBMCs isolated from YF17D-vaccinated volunteers display a mixed T-helper cell phenotype, with the induction of a mixed Th1/Th2 profile (159, 166). These studies provide a global description of the innate and adaptive immune responses induced by live attenuated (YF-17D) vaccine, showing the networking of the innate immune response that is required for the induction of effective long-lasting immune protection.

Systems approaches to understand molecular basis of virus-like particles (VLP)-HIV vaccines

Such approaches have been used to identify signatures of activation of a VLP-based HIV vaccine *ex vivo* on monocyte-derived DCs (MDDCs) as well as on PBMCs (167–170). Studies have been performed using a baculovirus-expressed HIV-VLPs (171), which have been shown to induce HIV-1-specific $CD4^+$ and $CD8^+$ T-cell responses and cross-clade neutralizing antibodies at systemic and mucosal level in immunized BALB/c mice (172–174).

Baculovirus-expressed HIV-VLPs induced maturation and activation of MDDCs from HIV-1 seronegative subjects, and this effect was partially mediated by the internal TLRs, TLR3 and TLR9. The HIV-VLP-activated MDDCs produced a pattern of cytokines indicative of both Th1 and Th2 pathways and induced primary and secondary responses in autologous human $CD4^+$ T cells in an *ex vivo* immunization assay (168). A specific pattern of cellular maturation and activation induced by HIV-VLPs was observed also on whole PBMCs from both HIV-1 seronegative and seropositive subjects, without further manipulation and differentiation to MDDCs (169, 170, 175). This analysis identified a number of HIV-1-seropositive subjects who showed a complete lack of maturation induced by HIV-VLPs in $CD14^+$ cells, confirming the relevance of this multiparametric approach to identify possible non-responders (169). HIV-VLPs induced a significantly increased production of Th2 cytokines only, strongly suggesting that specific Th1 adjuvants would be required for therapeutic effectiveness in HIV-1-infected subjects (169, 176).

In parallel, the baculovirus-expressed HIV-VLPs induced specific transcriptional profiles of genes involved in the morphological and functional changes characterizing innate and early adaptive immune response. This immune signature was observed in MDDCs (167) as well as in PBMCs from HIV-1 seronegative and seropositive subjects (170, 175). As described for the yellow fever live attenuated YF-17D vaccine, HIV-VLPs induced a molecular signature including several genes involved in innate sensing of viruses and antiviral immunity. Expression of proinflammatory mediators CXC-chemokine ligand 10 and IL-1α genes were found upregulated. Similarly, several genes were identified encoding innate sensing receptors (i.e. TLR2), transcription factors that regulate the expression of type I IFNs, IRF1, and STAT2.

The gene signature predictive of both humoral and cellular adaptive immune response included several genes. The CD83 and CD28 genes indicate a strong activation of the Th2 development and B lymphocytes (177–179). TNFRSF1B and TNFRSF6B are markers for T and B-cell activation (TNFRSF1B) (180) and for blocking the pro-apoptotic activity of the FAS-ligand (TNFRSF6B) (181). The TNFSF9 is a T-cell activation marker (182, 183), and CD40 is one of the key players in activation of both humoral and cell-mediated immune responses (184, 185).

These studies provide a global description of the innate and early adaptive immune signatures induced by non-replicating VLPs (HIV-VLPs) in MDDCs as well as PBMCs. Commonalities between these signatures and those induced by the live attenuated vaccine (YF-17D) suggest the possible identification of specific shared predictive gene expression meta-signatures with a broad application in vaccinology.

Polymorphisms and response to vaccines

Studies on polymorphisms of TLRs gene have enabled to recognize the influence of single nucleotide polymorphisms (SNPs) in TLRs on immune response to vaccines (186–188). In particular, associations between SNPs in both TLRs and the downstream intracellular MyD88 and MD2 signaling molecules with antibody and cellular responses to measles vaccination have been described recently (186). A SNP in the 3′UTR of TLR3 (rs5743305 at −976 bp of TLR promoter) has been identified and the heterozygous variant AT correlates with and low humoral and cellular responses in vaccinees. Similarly, the GA variant of a non-synonymous SNP also in the TLR3 gene was associated with lower antibody production. Heterozygous variants for two non-synonymous SNPs (Gly299Asp and Ile399Thr) in the TLR4 gene, already known to be associated with septic shock after infection with Gram-negative bacteria, premature birth, myocardial infarction, and allograft rejection (189), have been identified and associated with higher IL-4 secretion to the measles vaccine strain (186).

Associations between SNPs in genes of TLRs intracellular signaling molecules and the immune response to measles vaccine have been also investigated (186). A minor allele variant for a SNP in the 3′UTR of MyD88, the intracellular adapter molecule that signals for most of the TLRs, was found to be associated with a lower antibody response to measles vaccine, while intronic SNPs in TLRs and their associated intracellular molecule genes were significantly associated with variations in cellular immune responses to measles vaccine (186).

Similar results have been reported for rubella vaccination (187). Polymorphisms in promoter and intronic regions of TLR3 and TLR4 genes have been found associated with rubella virus-specific cytokine immune responses, such as IFN-γ, IL-2, TNF-α, and granulocyte macrophage-colony stimulation factor (GM-CSF). In particular, two SNPs in the TLR3 gene appear to be significantly associated with lower rubella IFN-γ secretion in an allele dose-related manner. Interestingly, the same promoter polymorphism (rs5743305, −8441 A > T) in the TLR3 gene, associated with rubella virus-induced GM-CSF secretion, is considered a risk factor for lower antibody and low lymphoproliferative responses to measles vaccine (186). This finding strongly suggests that this SNP in the TLR3 gene may play a more general role in viral immunity and represent a checkpoint for humoral and cellular immune responses to both measles and rubella vaccines.

In addition to genes of TLRs and their intracellular signaling, the same study described associations of polymorphisms in promoter and intronic regions of vitamin A and vitamin D receptor genes and their downstream mediators of signaling with different immune response to rubella vaccination (187). The influence of SNPs in the vitamin D receptor (VDR) genes

on proinflammatory immune responses to viral infection or live viral vaccination might be more general, considering that polymorphisms in the VDR gene are also associated with protection from HIV-1 infection (190). Polymorphisms in the TRIM5 gene have been associated with variations in rubella virus-specific immune responses, in accordance with recent findings on the role of the same TRIM5-gene SNPs in the immune response to retroviral (HIV-1) infection (191).

Concerning the pertussis (PT) vaccine, specific SNPs in the promoter region of the TLR4 gene as well as haplotype-tagging SNPs in genes of the TLR signaling pathway have been shown to influence the antibody response to vaccination (188, 192). SNPs in the TOLLIP gene were the most consistent in three independent analyses. The relevance of this association is due to the biological role of TOLLIP, which is a small protein that binds the activated IL-1 receptor type I (IL-1RI) complex as well as TLR2 and TLR4 complexes, coordinating optimal signaling through IL-1RI and TLR4 (193, 194). Furthermore, associations of SNPs in TIRAP and TICAM1 as well as IRAK3 and IRAK4 genes and immune response to PT vaccine have been also observed. In particular, TIRAP and TICAM1 belong to the TIR domain-containing adapters, including MyD88, that modulate TLR signaling pathways, while IRAK3 and IRAK4 are signal transduction mediators of the Toll and IL-1R families (188, 192).

These results give strong indications for the involvement of the TLR signaling pathway in the response to vaccination as well as for the cooperation of its genes in a functional interacting network. More important, they provide evidences that genetic variants are involved in the mechanisms underlying heterogeneous immune responses to vaccines and propose the possible identification of specific shared predictive polymorphisms with a broad application in vaccinology. All these studies need to be replicated in independent and larger cohorts to validate the findings, increasing the statistical association between SNPs and host immune response to vaccines.

These results indicate that a comprehensive analysis at the system-level would greatly facilitate screening for responsiveness to vaccines and an understanding of eventual failures in individuals enrolled in clinical trials. It will also guide the identification of optimal antigens and antigen formulations (i.e. adjuvanted antigens) to induce the sought cluster of genes and immune pathways leading to the required adaptive immune response.

Conclusions

In recent years, the field has seen a remarkable explosion of information about the components of innate immunity and their role in guiding and shaping the adaptive immune response. A significantly improved fund of knowledge has been accomplished regarding the PRRs, mainly the TLR family members, as well as the associated pathways and their role in the host responses upon exposure to a foreign antigen, in the form of vaccine or pathogen. In particular, many tiles have been added to the mosaic describing the molecular processing and recognition of antigens by cells of the innate immune system and how this will impact on the nature as well as the duration (immune memory) of the adaptive T and B-cell immune responses.

The development of safer but less potent recombinant vaccines, to minimize the possible biological risks linked to traditional inactivated or killed vaccines, is boosting mandatory studies aimed to improve their immunogenic and protective activity. In this regard, the use of TLR or non-TLR PRR signaling to boost vaccine efficacy is heavily explored and documented in several established vaccines. To this aim, systems biology holds considerable promise for discovery and new insights into complex networking processes such as

interaction between foreign vaccine antigens, innate immunity, and the downstream adaptive immune response.

Systems biology not only has the potential to accelerate the discovery of new regulators of innate immunity but also will provide more comprehensive insights into the kinetics of regulation at the transcriptional, protein–protein interaction, and post-transcriptional levels. All this information will be of high impact on vaccine development, providing molecular prediction markers of the immunogenicity of a vaccine, uncovering new correlates of vaccine efficacy, as well as guiding the design of new vaccine antigens or formulations. Moreover, such system-level approaches could permit the identification of vaccine responders versus non-responders, allowing a better immunological coverage of the licensed vaccines.

Recent pioneering studies describe results showing that system-level analyses may provide this invaluable information. Broader application of this strategy to other vaccines will definitely allow the possible identification and validation of common markers (i.e. gene signatures, SNPs) applicable to all or clusters of vaccines. The novel and improved knowledge on molecular components of the innate immune system together with the development of more potent high throughput computational analysis will lead to the switch from the ‘empirical’ to the ‘knowledge-based’ age of the vaccinology, enabling the development of even more successful vaccines for preventive as well as therapeutic intervention strategies.

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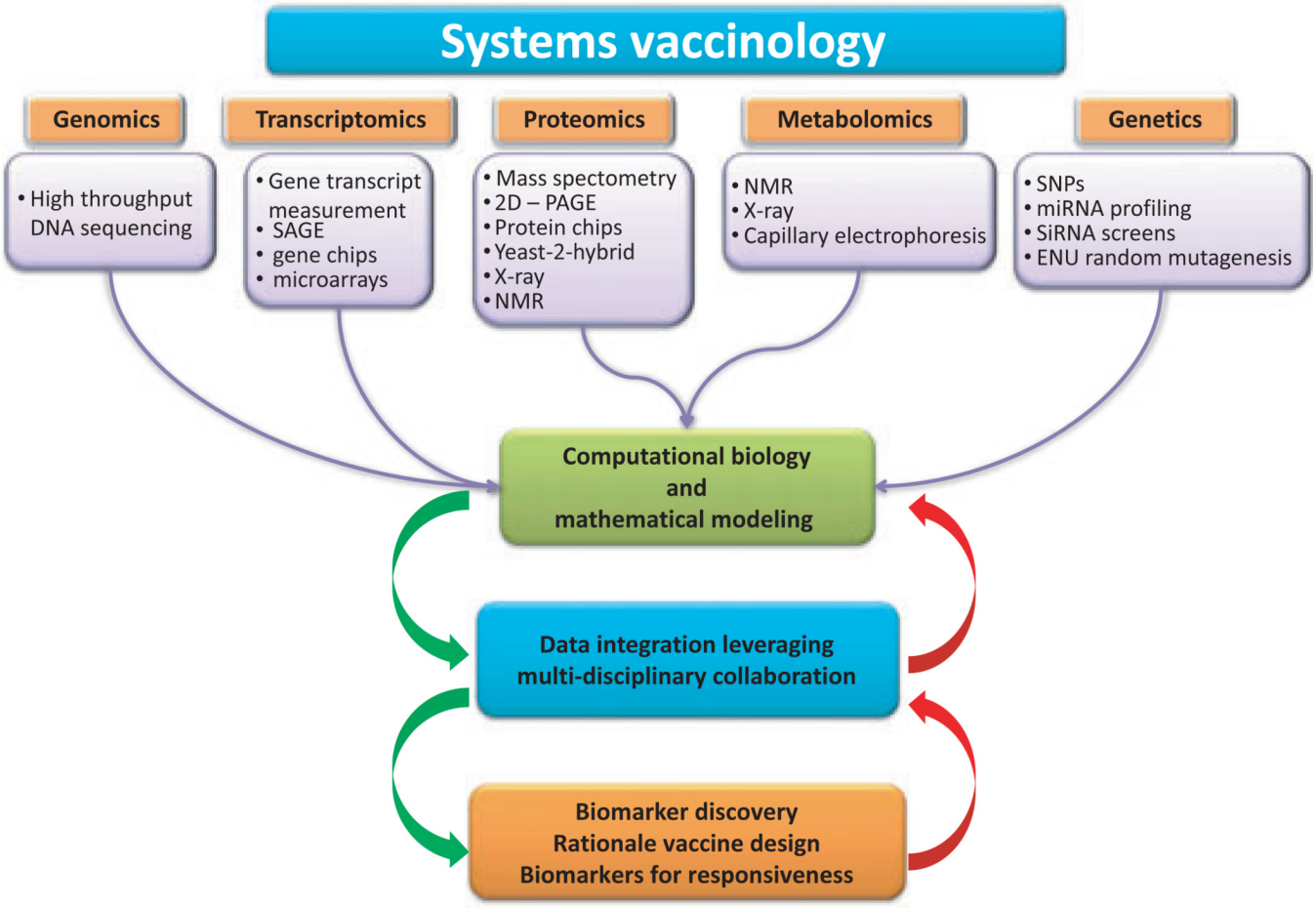

Fig. 1.
Systems biology approaches for vaccine studies interactions and the implications on translational research.