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Solving vaccine mysteries: a systems biology perspective

Lydie Trautmann & Rafick-Pierre Sekaly

Systems biology has emerged as a promising research strategy that can be applied to vaccine development. This approach can lead to the identification of new mechanisms and predictors of inactivated vaccine immunogenicity.

The search for vaccines against several incurable diseases, including acquired immunodeficiency syndrome, malaria, tuberculosis, and dengue fever, has largely failed, which highlights the need for new vaccine strategies[1]. In contrast to the empirical development of most efficacious vaccines, these diseases require a rational approach for directing vaccine responses toward different effector cell subsets of the immune system that vary with the nature or cellular tropism of these pathogens[2,3]. The lack of well-defined correlates of protection with most vaccines has been a major impediment to the successful development of new vaccines. The failure to identify such correlates has been mostly a consequence of the lack of assays that can measure integrated immune responses and have relied on the evaluation of a limited number of qualitative and quantitative features of effector adaptive immune responses. The efficacy of vaccines is also known to vary considerably in the human population depending on several environmental and genetic parameters, including age, nutrition and preexisting infections. In this issue of *Nature Immunology*, Nakaya and colleagues use systems biology to identify useful predictors of the efficacy of vaccines against influenza in humans[4].

Systems biology offers the unique possibility of analyzing the immunological network of complex events and interactions after vaccination[5]. This approach can help accelerate vaccine development by identifying predictors of immunogenicity and previously unknown mechanisms that underlie protective immune responses[6] (**Fig. 1**). Systems biology has been used to identify early gene signatures that can be used to predict the responses of B cells and $CD8^+$ T cells after vaccination against yellow fever[7–9]. Such studies have highlighted the importance of inducing a potent and diverse innate immune response for the generation of long-term protective adaptive immunity[7]. Most of these pathways would not have been identified by conventional immune response–monitoring strategies such as flow cytometry and analysis of cytokine production.

Despite the demonstrated success of systems approaches in predicting the immunogenicity of the vaccine against yellow fever[7,8] (a live replicating virus), the question of whether such approaches would also be useful in predicting the immunogenicity of inactivated vaccines (which do not replicate) has remained unknown. Furthermore, it has been unclear whether such approaches could be used to predict the immunogenicity of memory responses. The report from Nakaya *et al.* addresses these questions directly[4]. Using systems biology to analyze the innate and adaptive responses to seasonal influenza vaccination in humans, the authors define early predictors of a vaccine response and provide new insights about the mechanisms that underlie vaccine immunogenicity. Notably, this work describes a new comprehensive approach with which to tackle the challenge of vaccine development, not only because it defines new predictors of the inactivated influenza vaccine but also because it demonstrates and validates the systems biology approach as a powerful tool for predicting vaccine immunogenicity and discovering additional mechanisms of vaccine efficacy (**Fig. 1**).

This study compares immune responses induced by two highly efficient vaccines against influenza: the trivalent inactivated vaccine (TIV) and the live attenuated influenza vaccine (LAIV). The transcriptional signatures induced by TIV and LAIV are distinct, but both induce common innate immune response pathways, including an inflammatory response characterized by inflammasome and upregulation of the transcription factor NF-κB. In contrast, type I interferons, which are a common feature of the two live attenuated vaccines (LAIV and the YF-17D vaccine against yellow fever) and are potent inducers of innate immunity, are not observed after vaccination with TIV, a whole killed virus. Involvement of innate immunity in modulating the response to TIV is further confirmed by the positive correlation between gene-expression pathways associated with natural killer cell signaling early after vaccination (days 3 or 7) and hemagglutination-inhibition (HAI) titers at day 28. This highlights the fact that the innate immune response is diverse and is critical to the development of a strong adaptive immune response and also that the innate immune response can vary with the type of vaccine platform used. Notably, these different innate immune responses lead to protection. Such diversity of innate immune responses could never have been identified by conventional immune response–monitoring assays. Indeed, counting cells of the innate immune response or assessing cytokine profiles failed to predict the immunogenicity of the YF-17D vaccine[7]. Comparison of the innate immune responses induced by several other vaccines will provide not only a map of the common patterns induced by all successful vaccines but also the characteristics of particular vaccines that could be classified as either the type of vaccine formulation or the kind or immune response induced[3,6,10]. With this knowledge, the design of new vaccine regimens

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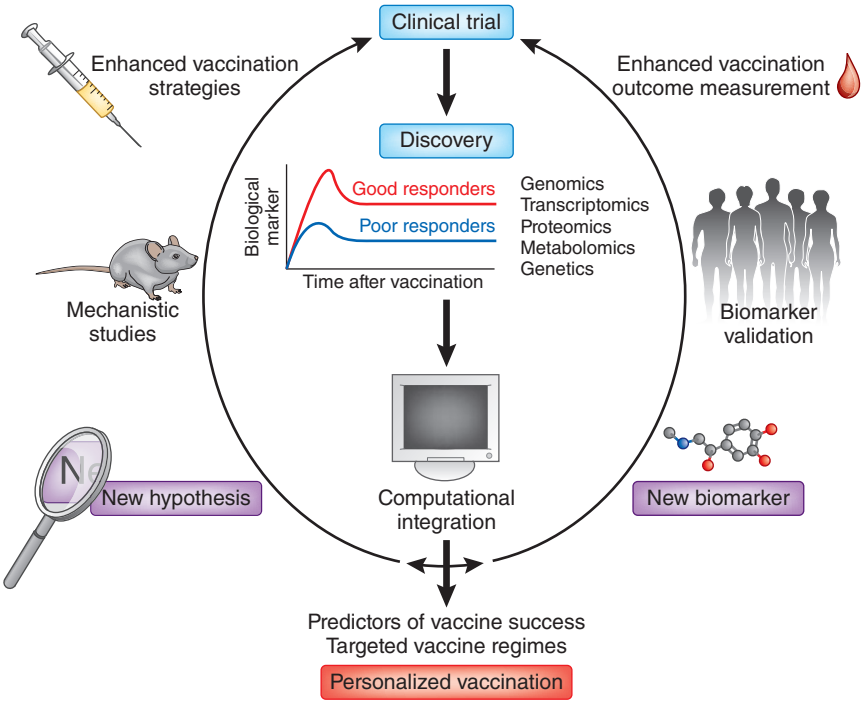

Figure 1 Systems biology approaches in the vaccine development. Licensed vaccines or new vaccines are tested in clinical trials in humans. Subjects are categorized as good and poor responders to a vaccine on the basis of biological markers. Samples collected are analyzed by one or more '-omics' approaches, and results are integrated by computational methods to generate meaningful data sets. Two outcomes can emerge from these bioinformatic analyses: the generation of new hypotheses that can be tested by mechanistic studies *in vitro* or in animal models and result in enhanced vaccination strategies, or the determination of new biomarkers that can be validated in other clinical trials and result in enhancement of the vaccination outcome measurement. Both of these arms will 'feed' new clinical trials that will follow the same path and, after many iterations of the cycle, lead to the development of better vaccination strategies and define better predictors of immunogenicity. This process might allow the generation of personalized vaccination.

and adjuvants could focus on recapitulating the common patterns required for vaccine efficiency and selective targeting of the specific type of response to be elicited.

Systems biology approaches can also provide insights into the molecular mechanisms that lead to vaccine efficacy. To reach this objective, however, it is necessary to assess the contributions of the various cellular subsets involved in the immune response that contribute to the overall signature in peripheral blood cells. Previous attempts aimed at deconvoluting these signatures have relied on the extrapolation of cell subset–specific gene-expression profiles from the frequency of these subsets[11]. Nakaya *et al.* propose an alternative solution for deconvoluting gene-expression profiles[4]. This approach is based on the meta-analysis of cell type–specific gene-expression signatures from publicly available microarray studies and does not require assessment of the frequency of specific immune-response cell subsets. Using this approach, the authors confirm that TIV induces the upregulation of genes mostly in B cells, especially antibody-secreting cells. In contrast, LAIV induces genes in T cells, monocytes and natural killer cells. These findings highlight the need to determine if gene-expression changes are due to changes in the distribution of specific cell subpopulations or to the *de novo* induction of gene expression in discrete subsets of cells. Hence, as illustrated by this study, such approaches also provide insight into the mechanism of action of vaccines and could guide the development of specific adjuvants.

To identify predictors of TIV vaccine immunogenicity, the authors use the DAMIP (discriminant analysis via mixed integer programming) model to train and test the predictors of two other independent cohorts vaccinated with TIV. By grouping subjects as 'good responders' (an increase of fourfold or more in HAI titers) and 'poor responders' (an increase of twofold or less in HAI titers), the authors find that gene signatures at days 3–7 that can be used to predict HAI titers at day 28 in the first cohort allow the prediction with 90% accuracy the immunogenicity of the inactivated vaccine in the other cohorts. Of note, some of the genes used to predict the magnitude of the TIV response, including *TNFRSF17* (a gene expressed during B cell maturation), have also been found to be predictors after vaccination with YF-17D.

This report illustrates all the steps and outcomes of systems biology approaches (**Fig. 1**). Starting from a comparison of two vaccines (TIV and LAIV) or of good and poor immunogenicity as estimated by HAI titers, the authors generate transcriptomics data that they further analyze by bioinformatics analyses. This study confirms the ability of such approaches to tackle complex network interactions that have never been studied before in humans in an unbiased way. For example, five members of the leukocyte immunoglobulin-like receptor family with high expression in monocytes and myeloid dendritic cells at day 3 after vaccination are found by the DAMIP model to be predictors of immunogenicity, which suggests a role for receptors of the innate immune system in regulating adaptive antibody responses. It is still unknown if the best predictors of vaccine immunogenicity would emanate from innate or adaptive immune responses. It is also possible that such predictors would be identified from the analysis of immune responses occurring in tissues or at mucosal sites. The ultimate goal would be to be able to predict if a particular subject would respond to a given vaccine by doing a simple cost-effective assay, such as quantitative PCR analysis of a drop of blood early after vaccination. The second outcome of this approach is the generation of unexpected hypotheses. This study finds that expression of the gene encoding CaMKIV, an enzyme involved in Ca^{2+} signaling, is inversely correlated with HAI titers induced by TIV at day 28. The role of CaMKIV in the regulation of antibody response has never been studied before[12]. The authors demonstrate that TIV induces phosphorylation of CaMKIV and further demonstrate a critical role for CaMKIV in regulating antibody responses, using mice deficient in CaMKIV.

The pioneering approach of Nakaya *et al.* has led to the generation of several new hypotheses that will pave the way for fundamental research focused on the identification of the mechanisms of protection induced by vaccines and will lead to the improvement of vaccination strategies[4]. Additional studies using similar systems biology approaches to identify determinants of vaccine and adjuvant efficacy will undoubtedly result in the discovery of previously unknown biological mechanisms by which various vaccines trigger and modulate the effector and memory arms of the immune response and thus revitalize immunology. Furthermore, the identification of signatures that can be used to predict vaccine immunogenicity will be of immense value in the early identification of people who respond suboptimally to the vaccine. This in turn will accelerate the pace of clinical trials and allow the design

of new vaccine strategies for incurable diseases and personalized vaccine regimens for immunocompromised populations.

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Erratum: Solving vaccine mysteries: a systems biology perspective

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In the version of this article initially published, the volume number for reference 2 was incorrect. The correct volume number is 12. The error has been corrected in the HTML and PDF versions of the article.