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Profiling immunity to HIV vaccines with systems biology

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Abstract

Purpose of Review—The recent modest success of the RV144 HIV vaccine trial in Thailand has shown that development of an HIV vaccine is possible. Designing a vaccine that achieves better protection, however, will require a more complete understanding of vaccine mechanisms of action and correlates of protection. Systems biology approaches enable integration of large datasets from a variety of assays and offer new approaches to understanding how vaccine-induced immune responses are coordinately regulated. In this review, we discuss recent advances in clinical trial design, specimen collection, and assay standardization that will generate datasets for systems analyses of immune responses to HIV vaccines.

Recent Findings—Several recently-published HIV vaccine trials have shown that different HIV vaccine prime/boost combinations can greatly affect the immune response generated, but mechanistic insights into their modes of action are lacking. Novel systems biology studies of efficacious, licensed vaccines provide a new template for analysis of HIV vaccines. To generate datasets appropriate for systems analysis, current HIV vaccine clinical trials are undergoing design modifications and increased standardization of specimen collection and immune response assays.

Summary—Systems biology approaches to HIV vaccine evaluation are driving new methods of HIV vaccine immune response profiling in clinical trials, and will hopefully lead to new improved HIV vaccines in the near future.

Keywords

HIV vaccine; systems biology; clinical trials; assay standardization

Introduction

The human immunodeficiency virus type-1 (HIV-1) epidemic has entered its third decade and has claimed over 25 million lives. Extensive animal and human studies have been conducted in the quest to find a successful vaccine to prevent or control HIV-1 infection (1). After numerous disappointing results, the field was buoyed by the findings of the recent RV144 trial, which showed that at least partial protective immunity against HIV can be achieved (2). Volunteer samples collected from this trial will allow the measurements of multiple immune responses and, for the first time, hope for understanding features that may have contributed to reduced acquisition of infection in vaccine recipients.

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Of potential help in this effort, the discipline of systems biology is designed to take a holistic approach to understanding a biological system by integrating analyses of many measurements of the system under different perturbations (3). Systems biology seeks a deeper understanding of biological processes and their interdependence, and produces models that closely reflect nature with the potential to predict biological responses. While the HIV research field has primarily focused on somewhat narrow assessments of immune responses to infection and vaccination that can be reliably measured with the samples available, expanding HIV vaccine analysis to systems biology approaches may help reveal the mechanisms of action behind successful vaccines such as those used in RV144 and will generate novel hypotheses that will drive a new era of rational HIV vaccine design (4). To collect the most comprehensive datasets for systems analysis, there is a need to gather immune response data using new HIV vaccine trial designs coupled with novel tools and assays to measure immune responses. Generating and integrating comprehensive datasets will be key for identification of the relevant response pathways to target with novel adjuvants and vectors for HIV vaccines.

HIV vaccine trials: turning points

Two HIV vaccine trials have recently attracted the world's attention for opposite reasons. In 2008, the results of the Phase IIb Step study were published, describing the efficacy and immune responses elicited to the Merck Ad5 HIV vaccine (5–6). Disappointingly, this regimen failed to prevent HIV infection or lower viral loads in individuals who became infected. Additionally, vaccinated individuals with preexisting Ad5 neutralizing antibodies became infected at a higher rate than individuals who did not possess such antibodies (7). Many groups have tried to determine the reason for this apparent increase in HIV acquisition risk, but to date no mechanism has been identified (5, 8–9). In contrast, the phase III RV144 trial, reported in 2009, demonstrated that a recombinant canarypox vector prime, subunit gp120 protein boost regimen had 31.2% efficacy against HIV acquisition (2). Initial immunogenicity analyses did not point to a clear reason for vaccine-induced protection, but extensive formal correlates analyses are still underway (10–11). The dramatically different and unanticipated results of these two trials, coupled with our lack of understanding of their mechanisms of action, highlight the need for additional integrated and more global approaches to HIV vaccine assessment.

Profiling in HIV vaccine trials to facilitate systems biology analyses

Many clinical studies of HIV vaccine products have been carried out over the past two decades (1, 12). Phase I and phase II studies typically assess safety and feasibility, as well as variations of the vaccine or inoculation regimen that affect immunogenicity. For example, two recently-published Phase I trials addressed the effect of DNA priming on a vector boost. One study (HVTN065) compared the effect of one or two doses of a DNA prime or a homologous vector prime, followed by two doses of a modified Vaccinia virus Ankara (MVA)-vectored boost on adaptive immune responses (13**). Interestingly, increasing the number of DNA primes produced a more T-cell biased response, whereas a repeated doses with the MVA vector was very efficient in eliciting antibodies. A second trial (HVTN068) compared recombinant HIV-1 DNA plasmid versus Ad5/HIV vector priming followed by the same Ad5/HIV boost and additionally examined the kinetics of the T-cell response (14**). This trial also found that a homologous prime/boost with repeated Ad5/HIV increased antibody responses, but that an HIV-1 DNA prime was more efficient at increasing T-cell responses. Interestingly, T-cell responses after the HIV-1 DNA vaccine alone were very low when measured by standard intracellular cytokine staining (ICS) assays, and the superior effect of the HIV-1 DNA in inducing higher magnitude vaccine-specific T cells was only observed after the Ad5/HIV boost. Taken together, these trials

(along with others, such as (15–16)) highlight an interesting role for HIV-1 DNA priming in influencing the outcome of the adaptive immune response. Standard T cell assays measuring intracellular cytokine expression have thus far failed to identify the mechanism of this effect, suggesting that alternative approaches will be necessary.

The immune response involves a complex interplay between different cell types and soluble factors that are induced post-vaccination and wane over time. In addition to the responses of T and B cells that are the focus of vaccine trial assessment, it has become increasingly clear in recent years that the early innate immune response has a large impact on shaping the adaptive response, making analysis of early responses to HIV vaccines important (17). Measurement of innate responses to a non-replicating viral vector-based vaccine need to occur soon after vaccination, and we have had success measuring significant effects on early systemic responses at days 1, 3 and 7 post-vaccination (Figure 1). For example, in one clinical trial with the replication-incompetent Merck Ad5 vector (HVTN071), we observed a dramatic reduction in lymphocyte counts in peripheral blood at 24 hours post-vaccination, with a return to baseline levels by day 3 (Figure 2). This highlights the importance of obtaining longitudinal samples that allow measurement of key responding cells at the proper time and location in order to gain an accurate picture of the on-going response. Similarly, in-depth longitudinal profiling of both peak and memory timepoints of B- and T-cell responses will give insight into the genesis and quality of the response that develops. As highlighted by the Step study, it is also important to understand the effect of vector immunity on the response, necessitating sampling after a boost dose for vectors without natural immunity in the population under study (Figure 1). Specimen collection over the course of the trial must include multiple specimen types to use in focused validated standard assays as well as in more global profiling, as described below. Finally, once key time points are identified, more targeted testing of a second, independent cohort of individuals can then be performed for validation and to increase the size of the datasets.

To sample vaccine-induced immune responses in human vaccine studies, two compartments of the body are reasonably accessible: the peripheral blood and mucosal surfaces (Figure 1). The peripheral blood carries the majority of immune cells traversing from lymphatic organs to sites of action and transports soluble factors secreted upon immune activation. Although soluble molecules such as antibodies and cytokines are mainly produced locally in the tissues by activated cells and become diluted in the blood, sensitive assays such as ELISA, multiplex bead array, or targeted proteomics such as selective reaction monitoring (SRM) are able to detect and quantitate these factors (18–23*, 24). Similarly, specific micro-RNAs (miRNA) found in the serum can inform of cellular changes with specific origins (25). In addition, antibody-based phenotyping and enumeration of cells by flow cytometry can be used to profile blood cell subtypes to measure temporal changes in immune cell populations (14**, 26). Therefore, subtle changes within different immune compartments are detectable through analysis of this compartment.

Quantitative and qualitative changes within mixed cell populations like PBMC yield important mechanistic insights, yet care must be taken to measure the contribution of rare populations which can otherwise be overwhelmed by more abundant signals. To address this concern, fluorescence-activated cell sorting (FACS) can be used to enrich for rare events and to boost signals emanating from these sub-populations (27**). In situations where there are too few rare cells to perform an assay with an isolated subpopulation, or where cellular sorting cannot be reasonably performed prior to analysis, new statistical approaches are being developed to de-convolute signals contributed by cellular subpopulations (28). One system that can be applied is the cell-type specific significance analysis of microarrays (csSAM), which uses global transcriptional measurements from bulk populations and

quantitative stratification of sub-populations by methods like flow cytometry to approximate the transcriptional profile of cellular subsets (27**, 28).

It is important to note that phenotypic and quantitative changes within cells of the peripheral blood do not necessarily imply underlying functional changes. It is therefore essential to combine phenotypic with functional data. Validated functional assays that analyze the cellular compartment of the peripheral blood include IFN- γ ELISpot and ICS. Much progress has been made in recent years in areas such as standardization of PBMC collection, processing and cryopreservation as well as performance of these validated assays (18–19, 29–31). Further efforts are now being undertaken to improve standardization of additional assays, such as proliferation, cytotoxicity, and viral inhibition (30, 32–34), for more quantitative, functional readouts in human trials. At the same time, technological advances are decreasing detection limits and allowing multiplexing of analysis parameters, ultimately reducing the amount of sample required for each analysis and increasing the information gathered. Global measurement techniques such as epitope mapping in vaccine-specific T cells (35, 36*) or transcriptional profiling in different specimens by microarray (27**, 37–40) enable identification of statistically significant changes at the cell population as well as at the molecular level, even in small-sized samples derived from mucosal sites.

Mucosal surfaces, the primary site of HIV transmission, are important endpoints for immune profiling (24, 41–43). Unfortunately mucosal sampling is much more challenging because of the invasiveness of collection procedures. Furthermore, cell yields are low and can be quite variable, composition of the sites can vary over time (e.g., over the menstrual cycle), and the tissues are complex and difficult to sample uniformly. The most easily-accessed specimens for HIV vaccine evaluation are mucosal secretions and fluids collected from the oral cavity or from bronchial, gastrointestinal and genital tracts. Once the samples are processed, assays comparable to those conducted with peripheral blood can be performed (Figure 1). Standardization of methods to collect, process and assay mucosal tissues has proven challenging, but many efforts now starting to address these issues (44–46).

Using systems biology to synthesize vaccine response measurements

While additional sampling and new assays provide a wealth of information about vaccine responses, methodologies are needed to synthesize the resulting data and identify results of interest, both within individual studies and across trials. Systems biology tools provide a robust framework upon which to make these comparisons, although assay standardization and careful quality control on the data acquisition side are essential for valid comparisons. After pre-processing, several statistical approaches can be applied for data analysis. Beyond testing for simple linear or non-linear correlations within the data, sophisticated machine learning algorithms that evaluate the importance of individual parameters for the prediction of a defined outcome (e.g., protective immunity) can be employed, such as has been used with yellow fever and influenza vaccines (3, 27**, 37–38). These analyses take into account the multifaceted nature of the immune system and enable the generation of new hypotheses for testing in future trials or in model systems.

The benefit of using systems biology approaches to correlate profiles of vaccine induced immune responses with proxies of protection against viral infections was recently demonstrated in three systems-based analyses of yellow fever and influenza vaccines published over the past years (27**, 37–38). Using integrated systems approaches, the studies were able to define early response signatures that predicted the immunogenicity of the vaccines and generated hypotheses leading to new mechanistic insight into the vaccines' modes of action. Early, innate immune responses were profiled over time in peripheral blood, using transcriptional profiling coupled with multiplex analysis of plasma cytokines

and chemokines. Then, computational methods were employed to identify innate signatures that predicted T-cell or antibody responses and these signatures were validated in other vaccine recipient cohorts. Finally, the authors were able to use the data to generate new hypotheses regarding the vaccine mechanisms of action to test *in vitro* or in mouse model systems.

The key difficulty facing the HIV vaccine field is our lack of understanding of the correlates of immunity to HIV, making identification of signatures that predict vaccine protection challenging. In addition, there is a strong need to more closely integrate human clinical trials of HIV vaccines with *in vitro* and animal model systems (47–51), so that predictions generated from clinical trials can feed back into systems that test hypotheses more quickly and can be dissected in detail. Information gleaned from mechanistic studies can then be translated into the design of new trials, assays, and vaccine candidates to allow collection of key specimens for analysis.

Conclusion

The application of systems biology to vaccine response profiling is beginning to open many new avenues of research that will enable a deeper understanding of vaccines currently in trials for HIV and other diseases and will accelerate rational vaccine development in the future (4). To achieve holistic profiling of vaccine responses in clinical trials, it is necessary to measure many facets of the immune response using both standardized and more novel assays, and to integrate data from responses profiled over time. New comprehensive clinical trial designs are beginning to address this issue by including profiling of innate immune responses in addition to adaptive responses, and by measuring responses at both systemic and mucosal sites using assays with diverse read-outs. As investigators generate large databases with profiles of different HIV vaccines, it will be vital to compare and contrast the immune profiles obtained to these vaccines with licensed and efficacious vaccines to identify new hypotheses for testing in future trials. In addition, it will be critical to form close collaborations between clinical researchers and investigators studying HIV vaccines using *in vitro* and animal model systems to additionally test hypotheses and dissect mechanisms of vaccine-induced immunity. We are optimistic that applying these new tools will bring about a new era of HIV vaccine development, paving the way for development of a highly efficacious HIV vaccine.

Key Points

- Systems biology approaches offer new avenues of investigation for understanding vaccine mechanisms of action
- New trial designs incorporating more extensive sampling will provide a greater picture of the immune response to vaccination
- Standardization of sampling and assays is critical to enable cross-study comparisons

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Figure 1. Clinical trial design allowing for collection of longitudinal data spanning the vaccine-induced early (innate) and late (adaptive) immune response

A. Scheme of a phase I clinical trial design using a prime/boost regimen to assess early and late vaccine-induced immune responses. Time points at which samples are collected are depicted (visit days). **B.** Overview of specimen collection from several time points illustrated in A. The assays that can be performed with samples collected from the respective collection sites are shown.

Figure 2. Dramatic changes in peripheral blood lymphocyte concentrations within the first week post-vaccination with the Merck Ad5 HIV vaccine (HVTN 071)

The robust early response emphasizes the importance of profiling immune cell populations from at the appropriate time and location. Each line represents the response of one individual (n=7). *p<0.05 with Hochberg adjustment from a statistical model assessing change in concentration over time.