Systems Biology and the quest for correlates of protection to guide the development of an HIV vaccine

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Abstract

Over the last three decades, a myriad of data has been generated regarding HIV/SIV evolution, immune evasion, immune response, and pathogenesis. Much of this data can be integrated and potentially used to generate a successful vaccine. Although individual approaches have begun to shed light on mechanisms involved in vaccine-conferred protection from infection, true correlates of protection have not yet been identified. The systems biology approach helps unify datasets generated using different techniques and broaden our understanding of HIV immunopathogenesis. Moreover, systems biology is a tool that can provide correlates of protection, which can be targeted for the production of a successful HIV vaccine.

Introduction

Vaccines have been successful in controlling and eradicating several infectious diseases including smallpox and poliomyelitis. However, vaccines tested to prevent chronic viral diseases (HIV, HCV) and other poverty related infections have mostly failed or elicited only a weak protective immunity [1]. Despite great advancements in the field of immunology, we still lack an understanding of pathogen-host interactions and of mechanisms that lead to the development and maintenance of protective immunity. Systems biology is an interdisciplinary approach that aims at defining the interplay between the cellular and molecular components of a biological system with the ultimate objective of identifying mechanisms associated to a variety of outcomes [2]. Systems biology has been increasingly used to analyze vaccine-host interaction and identify candidate correlates of immune protection triggered by vaccines. Definition of such correlates will prove a valuable asset in guiding the development of new immunization strategies. This review will focus on the current and potential future role of systems biology in the development of a vaccine against HIV.

Challenges in vaccine development in HIV

Although preventive strategies, such as circumcision and chemoprophylaxis, together with antiretroviral treatment have decreased the number of HIV infections [3], the development of a vaccine is still necessary for the long-term drug-free protection against the virus. Developing an HIV vaccine presents many challenges. Conventional vaccines have succeeded in protecting against one or

few virus strains, but the continuously evolving nature of HIV requires the development of a broad-spectrum vaccine capable of protecting against the several circulating HIV strains. Furthermore, the virus targets CD4+ T cells, responsible for orchestrating both the humoral and cellular immune responses that trigger viral control and clearance. Induction of a functional immune response at mucosal sites is required to block the infection at entry prior to virus seeding in lymphoid tissue. Correlates of protection, *i.e.* measurable parameters that characterize protective immunity to HIV [4], still have to be identified since there is no human model of spontaneous immunological recovery from infection and no efficient vaccine.

Early trials highlight the importance of antibodies in prevention of HIV acquisition

The first HIV vaccine to undergo a full-scale efficacy testing was a gp120-based vaccine (AIDSVAX) designed to induce neutralizing antibodies. Although the vaccine did induce antibodies that targeted HIV, the two trials (VAX003 and VAX004) showed no evidence of protection [5,6]. The failure of gp120 molecules to induce broadly neutralizing proved to be the most likely mechanism for the failure of this vaccine.

The STEP and Phambili trials were designed to test the concept that CD8 T cell responses induced by an Ad5 vaccine, which does not trigger the production of neutralizing antibodies [7-9], could elicit long-term protection from HIV disease.

This concept was based on studies of elite controller subjects who are HIV infected but maintain undetectable viral loads; this protection has been associated to potent and polyfunctional cellular immune responses [10,11]. This vaccine did not prevent infection, nor did it reduce levels of circulating virus in subjects who became infected, dampening the hope that a CD8+ T cell response would be sufficient to affect the course of infection. This was further confirmed in the HVTN505 trial where Ad5 vectors were combined with DNA vectors encoding of HIV genes including gp120; this vaccine combination failed to protect against HIV acquisition [9].

A clinical trial that involved a different combination of viral vector (canary pox) and gp120 (AIDSVAX B/E gp120) showed for the first time a decreased HIV incidence in vaccinated subjects [12]. The RV144 vaccine reduced the rate of HIV acquisition by 31.2% after three-year follow-up compared to the placebo group [12]. Few immunological parameters were found to be associated with risk of infection in subsequent studies. Binding of plasma IgA antibodies to HIV proteins was associated with a 54% risk of infection by HIV while binding of IgG antibodies to HIV proteins was associated with a 43% decrease in risk of infection [13]. Of note, the IgG antibodies did not show any neutralizing activity, suggesting a potential alternative role of antibodies in preventing the infection. More recently, the percentage of CD4+ T-cells able to produce multiple cytokines after Env stimulation [14] and host human leukocyte antigen haplotypes were also associated with vaccine-conferred protection [15]. A new trial testing a

similar pox vector with HIV clade C antigens will soon start in South Africa and will aim at improving the level of protection seen in RV144 by increasing the durability of the protective response; this should lead to the identification of novel correlates of protection.

Systems biology approaches to guide vaccine development: clues to biomarkers of response and predictors of efficacy

Vaccine efficacy can vary greatly depending on individual characteristics such as age, gender, ethnicity and immunological history. Unbiased systems biology approaches are critical in defining specific immune responses in different groups of vaccinees and directly correlate these immunological signatures with successful protection or failure.

Systems vaccinology has been applied in recent years to identify correlates of protection for vaccines against yellow fever (YF-17D), influenza and hepatitis B [16-18]. These first studies identified a gene that correlated with the antibody response in all the different vaccines (TNFRSF17) and suggested the possibility of detecting a “universal vaccine signature”. A first step towards clarifying common and different vaccine-elicited signature patterns of immunity was made recently by Li *et al.* that compared signatures derived from five different vaccines (MPSV4 and MCV4, TIV, LAIV and YF-17D). Three patterns of expression correlating with antibody responses from the different vaccines were identified: the polysaccharide response shared by the two meningococcus vaccines; a

primary antiviral response similar to that observed in studies of YF-17D; a protein recall response correlating with the TIV and the anti-DT response to MCV4 (MCV4-DT) [18]. While a systems analysis of samples taken at early time points after vaccination could allow the identification of correlates of response to vaccination, the analysis of samples taken prior to vaccination may identify predictors of response. This could permit better targeting of vaccine strategies and stratification of vaccinees beforehand, leading to personalized medicine. Moreover, our group has recently shown that the hyporesponse to hepatitis B vaccination in the elderly can be predicted prior to vaccination by the upregulation of interferon (IFN) and inflammation-related pathways [16]. Conversely, high CD4 numbers and elevated frequencies of memory B cells were associated to a potent response to this vaccine in the same population.

Similar studies should be applied in HIV infection. The identification of correlates of protection for HIV will greatly expedite the development of a successful vaccine. Systems approaches applied to non-human primates (NHP) models of HIV vaccination could be the key for designing and testing new vaccine candidates.

Vaccine studies in NHPs allow the identification of correlates of protection from acquisition

NHP have been widely used to model HIV pathogenesis and to test vaccination strategies [19]. Rhesus macaques (RM) infected with simian immunodeficiency

virus (SIV), the homologue of HIV, present a progressive disease that closely resembles human AIDS [20-22]. For this reason, RMs have been the most robust model for studying the protection from acquisition triggered by SIV/HIV vaccines.

Live attenuated SIV viruses (Δ nef) have been shown to provide near complete protection from challenge with homologous wild-type SIV strains [23]; however, the mechanisms of protection remained unclear. Using a systems biology approach to identify correlates of protection, Del Prete *et al.* showed that RMs vaccinated with high doses of multiple SHIV variants with different levels of attenuation elicited heterogeneous immune responses [24]. The live attenuated vaccines (LAV) that provided most protection were the least attenuated and most homologous to the challenge virus. Another study (using SIVmac239 challenge [25]) showed that the protective T cell response to the LAV in the lymph node may predict the outcome and is elicited almost exclusively in follicular helper T cells. This study highlighted the importance of effector T cells in tissues as the most important correlate of protection from SIV dissemination in LAV vaccinated NHPs.

Kurupati *et al.* tested a low-dose SIVmac239 LAV and challenged with SIVmac251 [26]. Genes associated with dendritic cell functions, macrophage maturation, chemokine receptors among others were found to be associated with protection in macaques while administering the LAV. Belisle *et al.* [27] included RM immunized with a SIV DNA vaccine alone or in combination with

CCL5/RANTES, using challenge of SIVmac251. This analysis showed that IFN pathways identified different transcriptional profiles between treatment groups. Another study published by Selinger and colleagues [28] used RM vaccinated with irradiated gp96^{SIV}Ig cells transfected with Rev-Tat-Nef, Gag and gp160 with/without a gp120 boost and challenged with low-dose SIVmac251. Transcriptional profiling showed that delayed viremia was associated with macrophage-related inflammation and enrichment in activation of IFN-regulatory factors and IFN after challenge. Together with Del Prete's results these studies indicate an important role of the IFN pathway in protection of infection. IFN pathways have already been tested *in vivo* by administration of IFN- α 2 in acute phases of infection [29].

The aforementioned studies and others have provided useful information, which may help pave the way into the identification of biomarkers that can guide the development of an efficacious HIV vaccine.

Meta-analysis identify T cell activation and inflammation as important correlates of protection across SIV vaccines

Systems biology approaches, which can merge diverse sets of observations, are vital for the identification of correlates of protection. In order to uncover common signaling pathways associated with protection by SIV vaccines, we performed a meta-analysis of publically available transcriptomic datasets. To that end, the GEO and ArrayExpress databases were queried with the keywords "SIV

vaccine". The meta-analysis was restricted to studies in which RM were vaccinated and subsequently challenged to assess vaccination efficacy. The majority of the retrieved transcriptomic studies were performed on blood. Hence, studies that looked at other tissue types were excluded from further analysis. Our analysis was focused on the gene expression profiles obtained *ex-vivo* before challenge and we excluded stimulated or post-challenge samples. The three datasets that passed the filtering criteria are summarized in **Table 1** and reviewed in the previous paragraphs.

The meta-analyses performed allowed the identification of a set of 13 pathways associated with protective immunity across the five SIV vaccine formulations. The signaling pathways can be separated in four immunological processes (Figure 1): T cell activation, monocyte signaling and B cell memory processes are positively associated with protective immunity while inflammation is negatively associated with protective immunity. Among the positively associated processes, T cell activation is the most represented in the pathway enrichment results. The T cell activation process encompasses several genes up-regulated in naive T cells compared to unstimulated dendritic cells such as CD8B. CD8B encodes for the CD8 antigen that identifies cytotoxic T cell and was positively correlated with protection by SIV vaccines (Pearson correlation (r)=0.407, t-test p-value (p)=0.0481). The second most represented immune process among the pathways associated with protective immunity was monocyte signaling. This

immune process includes genes upregulated in monocyte compared to naïve T cell [30].

Among those genes, *IL-6R* had the highest correlation with protection by SIV vaccines ($r=0.569$, $p=0.00438$). *IL-6R* is a gene coding for a subunit of the interleukin 6 (IL-6) receptor, which is implicated in IL-6-regulation of cell growth and differentiation during immune response. B cell response is also associated with protective immunity. Genes in this immune process are up-regulated in the Ig isotype switched memory B cells versus plasma cells comparison [31]. Among the genes significantly correlated to SIV vaccine protection and part of the B cell response pathway, we can note the presence of NF- κ B1 ($r=0.466$ $p=0.0216$) and HIVEP2 ($r=0.421$, $r=0.0407$). Both NF- κ B and HIVEP2 are implicated in the generation of memory Th1/Th2 cells that are important for the induction of B cell memory [32]. Inflammation was associated with poor protective immunity. A representative of the genes included in the inflammation response module was SORBS2 a type-I IFN induced gene negatively associated with protection ($r=-0.229$, $p=0.282$). Altogether these results highlight the importance of system biology approaches in defining correlates of protection to different vaccines. The bioinformatic tools allow a thorough unbiased investigation of vaccine-induced responses.

Conclusions

There is a need for an HIV vaccine that not only stimulates the innate compartment to present antigens adequately but also that induces both arms of the adaptive response. Recent studies from our group and others have shown that the pre-vaccination state of an individual influences the response to vaccine. This could lead to the development of more complex strategies that involve immune modulators targeting inflammation that impedes on the response to vaccination. Perhaps the best and more counterintuitive example stems from studies of Mannick *et al.* who showed that rapamycin, known for its capacity to prevent graft rejection, can now be used to improve the response to vaccines in elderly [33]. System biology approaches have been critical in defining novel pathways which interfere with the immune response to vaccination paving the way to novel interventions that can enhance the response to vaccination in immunocompromised subjects including elderly, subjects undergoing myeloablative interventions and subjects in developing countries. The addition of other systems-wide approaches including analyses of the microbiome and of the metabolome, as well as the identification of epigenetic factors associated to strong vaccine responses will allow the identification of novel correlates of protection. More importantly, these new systems-wide studies will lead to a better definition of the mechanisms that lead to vaccine mediated protection in all categories of subjects (Figure 3).

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FIGURE LEGENDS

Figure 1. List of pathways associated with protective immunity across five SIV vaccines. The Selinger [28], Belisle [27] and Kurupati [26] transcriptomic datasets were used to perform a meta-analysis seeking pathways correlating with protection and elicited by the different SIV vaccine regimens. Briefly, the chips of the Selinger dataset were quantile-normalized and the probe intensities log₂ transformed for variance stabilization. For each macaque, the pre-vaccination gene-expression was subtracted to the week 32 gene-expression (week 32 is the time point before challenge). Then, for each gene, the R package LIMMA was used to fit a linear model with the gene-expression as dependent variable and the number of SIV infection challenges as independent variable. Macaques treated with SIVgp16 without gp120 boost were excluded from this analysis because the challenge information was not available. A moderated t-test was used to assess the statistical significance of the association of the gene-expression and the number of SIV infection challenges. The chips of the Belisle dataset were quantile-normalized and the probe intensities log₂ transformed for variance stabilization. The SIV pol stimulated samples were excluded from further analysis. For each gene and each vaccine, the R package LIMMA was used to fit a linear model with the post-52 week (pre-challenge) gene-expression as dependent variable and the log viral load at set point (measured > 80 days post-challenge) as independent variable. A moderated t-test was used to assess the statistical significance of the association of the gene-expression and log viral load at set point. The raw FASTQ files of the Kurupati dataset, were trimmed for poor Q-score nucleotide calls, aligned to the rhesus macaque transcriptome (Ensembl Mmul_1 build) and the mapped reads counted. The counts were normalized using the Trimmed Mean of M-values approaches. For each gene and each vaccine, the R package edgeR was used to fit a generalized linear model with the gene expression as dependent variable and the time point plus the number of SIV challenge to infection (i.e. first positive viral load) as independent variables. A likelihood-ratio test was used to assess the statistical significance of the association of the gene-expression and the number of SIV

infection challenges. For each vaccine, Geneset enrichment analysis (GSEA) was used to assess the enrichment of Molecular signaling database-curated genesets (C2) and immune related genesets (C7) among genes associated to protective immunity. To that intent, genes were ordered from the most positively correlated to protection to the genes that most negatively correlated to protection as assessed by their t-statistic or likelihood-ratio. GSEA assessed the strength (NES enrichment score) and the statistical significance (p-value) enrichment of each genesets in the top (or bottom) of the rank-list. To identify genesets enriched across vaccines a Fisher combined p-value was calculated based on the GSEA p-values obtain on each separate vaccine. At a stringency cutoff of combined p-value ≤ 0.05 , 13 genesets were identified as being shared across vaccines. Each identified genesets is presented as a circle. Each section of the circle was colored by the NES calculated for one of the five vaccines. A section in red represents a geneset enriched among genes positively correlated to protection while a section in blue represents geneset enriched among genes negatively correlated to protection. Genesets sharing genes in common (grey edges) as assess by Jaccard index $> 1/10$ were regrouped into a same module representing a distinct immune process (depicted in colored area). Three immune process, B cell response (brown), monocyte expression (green) and T cell activation (purple) were positively associated with protective immunity. One biological process, inflammation response (orange), was associated with a poor protective immunity.

Figure 2. Gene-expression signatures associated with protection across vaccines. The set of genes contributing the most to significant enrichment of the gene sets presented in Figure 1 were identified by performing a leading edge analysis. For each leading edge gene, the t statistics reflecting their association with protection was combined across vaccines using the procedure proposed by Weir and converted to p-value. Leading genes with a t-test p-value ≤ 0.05 are presented on this heatmaps. The scaled-expression (mean of zero, standard deviation of 1) of each gene (rows) in each dataset is presented using a blue-

white-red color gradient. The columns represent the sample. The time point post-vaccination (all pre-challenge, bottom bar), the treatment received by each animal (middle bar) and the protection variable (top bar in green gradient) are presented above each sample. Genes were ordered by their membership to each immunological process. Samples were ordered by the increasing gene-expression of the gene positively associated with protection.

Figure 3. Systems vaccinology approach for the search of potential vaccine candidates. Systems biology and bioinformatic tools allow the integration of different datasets derived from clinical trials and animal model-based studies for the generation of new hypothesis and identification of correlates of protection. These can then be targeted for a successful HIV vaccine.

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* This study showed that B cell signaling is associated with strong responses to hepatitis B vaccination. In contrast, inflammation was associated with poor responses to hepatitis B vaccination. Using a systems biology approach, the data generated in this study indicated that 2/3 of responses to vaccine in elderly individuals can be predicted by their pre-vaccination gene signature.
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** This study compared the immunogenicity and protection from repeated low dose of two different SIVmac251-based vaccines. They showed that SIVΔnef provided some protection, while AdHu5gag vaccine was ineffective. They identified gene expression signatures associated with delayed infection, and their associated pathways. The data generated in this study was used in the meta-analysis performed in this review.
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** This study tested a RANTES-adjuvanted DNA-based vaccine in RMs. The use of RANTES induced a better suppression of viral replication during chronic infection, and had a better vaccine-induced CD8 T cell

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** Using whole blood-transcriptional profiling, this study identified genetic signatures associated with strong IFN responses after challenge. This study also showed that prime-boost vaccination lowered the probability of infection in a time-consistent manner throughout several challenges. The data generated was used in the meta-analysis performed in this review.

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** This study tested *in vivo* the effects of IFN by manipulation of IFN-I signaling by IFN-1 blockade and administration of IFN- α 2 in RMs. Both treatments induced expression of antiviral genes during acute phase of infection, but continued treatment caused desensitization and decreased antiviral gene expression. These data show that the timing of IFN-induced responses during acute infection affects the course of disease and outcome.

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* This study showed that inhibition of the mTOR pathway using RAD001 enhanced the response of elderly individuals to influenza vaccination, as well as PD-1 expression on both CD4 and CD8 T cells. These results raise the possibility that using inhibitors of the mTOR pathway can prevent immunosenescence in the elderly.

Table 1. List of transcriptomic datasets used in the meta-analyses of SIV vaccines studies.

Dataset	Kurupati [24]	Selinger [26]	Belisle [25]
Vaccine	SIVmac239 AdHu5gag	SIV gp96+gp120	SIV DNA SIV DNA+RANTES
Species	Rhesus macaque	Rhesus macaque	Rhesus macaque
Number of donors	5+6	12+12+12	6+6+6
Tissue	PBMC	Blood	PBMC
Time point (weeks)	0 (pre-vax), 30	-2 (pre-vax), 32	52 (no pre-vax)
Challenge	Low-dose rectal challenges with SIVmac251	Low-dose rectal challenges with SIVmac251	High-dose rectal challenges with SIVmac251
Correlate of Protection	First positive viral load	Number of challenges to infection	Log viral load at set point (> 80 day post-challenge)
Accession number	E-MTAB-1130	GSE59068	GSE35816
Platform	Illumina RNA-Seq	Agilent one-color	Agilent one-color

PBMC, peripheral blood mononuclear cells; pre-vax, pre-vaccination.

A. Enriched in protected animals

B. Enriched in non-protected animals

Selinger et al.

Belisle et al.

Kurupati et al.

SYSTEM VACCINOLOGY