Novel approaches, including systems biology, to HIV vaccine research and development: Report from a Global HIV Vaccine Enterprise Working Group

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A. INTRODUCTION

The Global HIV Vaccine Enterprise convened a two-day workshop on August 10-11 2009, at the Fred Hutchison Cancer Research Center offices in Seattle, WA, to discuss the application of novel approaches, including systems biology, to HIV vaccine research and development. The goals of this Working Group were to identify key scientific issues and opportunities that have emerged since the Enterprise Scientific Strategic Plan[1] was published in 2005, and to make recommendations to Enterprise stakeholders.

Earlier this year, for the first time in the more than three decades since the discovery of HIV, a candidate HIV vaccine was shown to protect a modest proportion of volunteers[2] from HIV acquisition, revitalizing a field that has been humbled by repeated failure. A large number of questions has been raised by this trial, the most important being what caused this modest effect on protection from acquisition. The nature of the immunological parameters that gave rise to this result is unknown, highlighting the urgent need to understand the pathways that must be activated to elicit the immune responses necessary to prevent and/or control HIV infection. We will argue here that the new tools of systems biology, when combined with current approaches, will be transformative in understanding the molecular networks underlying the immune response to HIV infection and will enable strategies to re-engineer these networks to generate protective immunity[3,4].

It is important to recognize the critical interplay between technology development and biology; when biological questions cannot be answered with current technologies, new ones need to be developed which, in turn, open new areas of biological inquiry. Novel systems biology techniques allow integration of hierarchical levels of information, leading to a deconvolution of the complexity of biological systems[5]. These approaches include "omics" measurements, such as genomic, transcriptomic, proteomic, metabolomic and lipidomic technologies. However, meaningful analyses of large sets of data in a multitude of formats also require substantial computational technologies to facilitate data visualization, model building and ultimately the capacity to simulate the necessary immune responses *in silico*. Therefore, deployment of systems biology approaches in HIV vaccine research requires the concerted efforts of HIV vaccine researchers, experts in systems biology and computational specialists. Initial attempts at applying systems level approaches to vaccine development show promise, particularly in defining molecular signatures induced early after vaccination that correlate with and predict the later adaptive immune responses in humans[5–7]. Furthermore, systems approaches are beginning to be applied to understanding the mechanisms of action of vaccine adjuvants and vectors[8,9]. Given the preliminary success of these efforts, the Working Group has recommended implementation of a coherent suite of structural, organizational, cultural and funding approaches to catalyze integration of novel technologies with HIV vaccinology. The Working Group also discussed the need for development of new technologies and for attracting and training of new talent. This report summarizes the discussions of the Working Group, evaluates current gaps, and proposes recommendations for future directions.

B. CHALLENGES, OPPORTUNITIES AND THE WAY FORWARD

Classical approaches to vaccine development aim to understand a specific property of a pathogen or a specific immunological response that might be exploited to develop a vaccine capable of eliciting long-lived protection against that pathogen. However, the intrinsic complexity of the immune system presents a barrier to understanding how a coordinated and integrated immune response can lead to protective immunity against HIV. More recently, new approaches have been developed that allow exploration of the complex, interconnected networks involved in immune responses to viruses such as HIV. These approaches have the potential to transform the field from the search for a single correlate of protection to identification of the multifactorial signatures associated with immunological protection and provide clues to protective mechanisms.

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In the discussions of this Working Group, four priority areas were identified that are critical to harness and integrate novel approaches and technological advances into HIV vaccine research and development (**Table 1**).

Priority 1: To collect and integrate quantitative data on human immunity in normal, vaccinated, and infected individuals and the process of HIV transmission and dissemination

Clinical trials and human cohorts provide invaluable insights into human immunology, immune responses to vaccination and processes of HIV transmission and dissemination. Thus, we propose that "omics" technologies be actively applied to collect data on the human immune system, especially in cases where it is perturbed by either vaccination and/or infection.

To facilitate collection and integration of data from different studies, a harmonized minimal set of clinical, immunological, and virological parameters needs to be identified and adopted by the field (including protocols, data upload strategies and ontologies). Whether stored in a single database or in a collection of databases, the data from the diversity of assays, time points and sources needs to be annotated, inter-related, and made available for analyses in ways that will allow comparison of data and assays.

We recognize that some aspects of HIV infection and early events following vaccination are not amenable to detailed and systematic analysis in humans using current technologies. Therefore, we need to both develop new and make better use of existing model systems (non-human primates (NHP), mice, *ex vivo* human tissue and others). Insights gained from these model systems will help to identify mechanisms involved in immune protection in humans.

Recommendations

This priority involves a two-step implementation strategy. First, scientists from within and outside of the HIV vaccine field should be brought together to define and agree on a number of issues which include: (i) appropriate population cohorts for both immunological and virological studies; (ii) the minimal harmonized set of measurements required for integrative analysis that allows comparison between different studies; (iii) common regulatory norms for informed consent and for data and specimen sharing, ensuring protection of volunteers involved; and (iv) data upload strategies, architecture, ontologies and user-friendly interfaces. Second, Enterprise stakeholders need to establish a process for coordinated and systematic data and sample collection, storage, and distribution to ensure prompt access for projects serving a larger aim of HIV vaccine research. This coordinated approach could be assisted through a central facility, a contract research organization with global reach, or a laboratory responsible for quality assurance for each of the agreed upon various measurements, with a transparent process to ensure access to the samples and database(s).

Priority 2: To create a global data sharing infrastructure for data storage and analysis

Large amounts of data are generated in HIV vaccine research. Therefore, rapid data sharing is essential to take full advantage of this large amount of information. Furthermore, systems biology approaches, which have the potential to accelerate multidisciplinary integrative analysis, depend on a culture of data sharing. For example, integration of genomics and immunological data will provide insights into the contribution of host genetics that might underlie population variability of vaccine-induced responses. The field needs to craft a standard data and reagent sharing agreement that is achievable and broadly supported. In addition, appropriate infrastructure will be necessary for data deposition, storage, access, and analysis. Multiple databases already exist in the HIV vaccine field and the challenges in making them accessible to a broader scientific community are numerous. They include connecting databases in a way that allows integration of diverse data types, providing access to data without compromising privacy of subjects and developing user-friendly tools and interfaces that allow data visualization and analysis.

While open data sharing is ideal from the perspective of the scientific community, it should be recognized that clinical research is always connected to the rights and privacy concerns of study volunteers. Clear transparent guidelines need to be developed and com-

Table 1 Summary of priorities and recommendations

Priority 1: To collect and integrate quantitative data on human immunity in normal, vaccinated, and infected individuals and the process of HIV transmission and dissemination.

- Scientists from within and outside of the HIV vaccine field to define:
  - (i) appropriate population cohorts for both immunological and virological studies;
  - (ii) the minimal harmonized set of measurements required for integrative analysis that allows comparison between different studies;
  - (iii) common regulatory norms for informed consent and for data and specimen sharing, ensuring protection of volunteers involved; and
  - (iv) data upload strategies, architecture, ontologies and user-friendly interfaces
- Funders and institutions need to establish a process for coordinated and systematic data and sample collection, storage, and distribution to ensure prompt access for projects serving a larger aim of HIV vaccine research.

Priority 2: To create a global data sharing infrastructure for data storage and analysis.

- Support a cultural shift in the field to foster data sharing by creating co-ordinated, integrated, harmonized and quality-controlled databases (including long-term funding for technological support and computational infrastructure).

Priority 3: Promote iterative rational vaccine design and evaluation by development, dissemination, and implementation of novel computational, systems biology, and technological tools in pre-clinical and clinical studies.

- Use a combination of *in silico*, *in vitro* platforms, NHP models and human trials to promote systematic evaluation of candidate vaccine components and immune engineering strategies.
- Invest in development and commercialization of technologies identified in the priority.

Priority 4: Build capacity by attracting and developing talent, including scientists from resource-limited settings and from outside the field.

- Establish and fund long-term programs that bring together systems biologists, vaccinologists, immunologists, virologists and epidemiologists.
- Create mechanisms to strengthen career development pathways for young and early career investigators.
- Invest in capacity building in resource-limited countries that is focused on long-term solutions that involve local financial and organizational support.

municated effectively to participating communities guaranteeing protection of their rights while maximizing public benefit. Field-wide adoption of these guidelines will contribute to building community trust and will greatly facilitate our ability to share, integrate and analyze datasets collected by different research groups.

Recommendation

Support a cultural shift in the field to foster data sharing by creating co-ordinated, integrated, harmonized and quality-controlled databases (including long-term funding for technological support and computational infrastructure). A working group representing different Enterprise stakeholders and experts could discuss and agree on technological, regulatory and ethical issues surrounding creation of such relational databases.

Priority 3: Promote iterative rational vaccine design and evaluation by development, dissemination, and implementation of novel computational, systems biology and technical advances in pre-clinical and clinical studies

Rational vaccine design and testing will benefit from the development and integration of novel computational, systems biology and technical advances in pre-clinical and clinical studies. Systems biology approaches depend upon the iterative process of developing computational models and testing of these models in situations where the modeled system can be perturbed. Thus, the focus of these efforts should be on modeling the interplay between viral infection and immune responses, as well as on analysis of timing, location, and extent of vaccine-induced immune responses. When applied in this manner, computational approaches will give valuable insights into the mechanisms of infection and protection, complementing and building on the knowledge gained in pre-clinical and clinical studies. Additionally, these novel tools will be useful when designing and performing integrative studies to re-engineer immune responses for the desired outcome in different *in vitro* and *in vivo* models.

In addition to efforts in computational and systems biology approaches, it is critical to develop and disseminate novel tools and assays to answer questions that cannot be readily addressed by current approaches. We need to support the development and utilization of high-throughput technology platforms, novel cellular and molecular assays for immune cells and mediators, ultra-high-throughput sequencing, single cell fluidic analyses, and non-invasive/minimally-invasive imaging technologies for analysis of *in situ*/*in vivo* responses to HIV infection and to vaccination. Also, we stress the importance of developing robust technologies for sample collection and phenotype measurement applicable in resource-limited settings, including barcoding, RNA, protein, lipid and cytokine analysis and appropriate sample storage to ensure data quality and standards.

Recommendation

Use a combination of *in silico*, *in vitro* platforms, NHP models and human clinical trials to promote systematic evaluation of candidate vaccine components and immune engineering strategies. This recommendation could be implemented by engaging systems biology approaches in the design of clinical trials, incorporating "omics" approaches to collect comprehensive data on vaccinees and controls, and providing inter-related data to the scientific community for exploration and generation of novel hypotheses. Investments in development and commercialization of technologies identified in the priority above will also be required.

Priority 4: Build capacity by attracting and developing talent, including scientists from resource-limited settings and from outside the field

Ensuring that HIV vaccine research benefits fully from the ongoing revolution in biomedical research requires input from researchers outside the field, technology developers, and computer modelers, as well as active involvement from scientists in endemic regions. The HIV vaccine research field is considerably more collaborative and multidisciplinary now than in 2005, when the first Enterprise Plan was published; however, the Working Group members felt that even more effort is needed to embrace the priorities and accomplish the recommendations in this Report. The field needs to harness expertise from other fields of biomedical research, as well as to nurture a new generation of scientists. In addition, sustainable capacity for HIV vaccine research in resource-limited countries needs to be built by engaging local government and industry as full partners in HIV vaccine research and development. Governments and industry should be encouraged to provide long-term commitments to develop and maintain research infrastructure, create defined career paths, and ensure that young and early-career investigators receive the mentorship and support they need.

Recommendation

Establish and fund long-term programs that bring together systems biologists, vaccinologists, immunologists, virologists and epidemiologists. Create mechanisms to strengthen career development pathways for young and early career investigators. Invest in capacity building in resource-limited countries that is focused on long-term solutions that involve local financial and organizational support.

C. CONCLUSIONS

HIV vaccine research and development is at a crossroads, and there is a critical need for new approaches to build on recent advances in the field. A systems biology approach to HIV vaccine research and development aims to integrate quantitative information on the biology, immunology and epidemiology of HIV infection and transmission into predictive models which support the rational design and testing of novel vaccine strategies. The multidisciplinary nature and iterative approach of systems biology distinguishes it from the linear discover-develop-deliver pipeline of empirical vaccinology. In addition, exploiting new opportunities provided by systems biology is contingent on engagement of a considerably wider research community with different skills and ideas. Systems biology offers a potentially fruitful strategy that can make a difference at a number of levels, including identification of the signatures of immune protection, identification of novel adjuvants and vectors, and the scientific insights that will enable rational vaccine design.

1. Coordinating Committee of the Global HIV/AIDS Vaccine Enterprise The Global HIV/AIDS Vaccine Enterprise. Scientific Strategic Plan. *PLoS Med.* **2**, e25 (2005).
2. Rerks-Ngarm, S. *et al.* Vaccination with ALVAC and AIDSVAX to Prevent HIV-1 Infection in Thailand. *N. Engl. J. Med.* 10.1056/NEJMoa0908492 (2009).
3. Ideker, T., Galitski, T. & Hood, L. A new approach to decoding life: systems biology. *Annu. Rev. Genomics Hum. Genet.* **2**, 343–372 (2001).
4. Aderem A. Systems biology: its practice and challenges. *Cell* **121**, 511–513 (2005).
5. Querec, T.D. *et al.* Systems biology approach predicts immunogenicity of the yellow fever vaccine in humans. *Nat. Immunol.* **10**, 116–125 (2009).
6. Gaucher, D. *et al.* Yellow fever vaccine induces integrated multilineage and polyfunctional immune responses. *J. Exp. Med.* **205**, 3119–3131 (2008).
7. Pulendran, B. Learning immunology from the yellow fever vaccine: innate immunity to systems vaccinology. *Nat. Rev. Immunol.* **9**, 741–757 (2009).
8. Mosca, F. *et al.* Molecular and cellular signatures of human vaccine adjuvants. *Proc. Natl. Acad. Sci. USA* **105**, 10501–10506 (2008).
9. Monaco, A. *et al.* Molecular immune signatures of HIV-1 vaccines in human PBMCs. *FEBS Lett.* **583**, 3004–3008 (2009).