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Conference report

"*In vitro* systems to characterize the immune response to HIV-1 and HIV-1 vaccine candidates", NIAID Workshop Report, Bethesda, August 4, 2010

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ABSTRACT

Although clinical trials are the ultimate way to prove vaccine safety and efficacy, the complexity, cost and time required to develop a product to enter human trials demand a serious, long-term investment. Lack of knowledge on immune correlates of protection from HIV infections makes investments in HIV vaccine research significantly risky. Preclinical testing of HIV vaccines is routinely carried out in non-human primate models however these studies have a significant cost and their predictive value is still questionable. The potential value of screening new HIV-1 vaccine candidates on human cells and tissues via high throughput *in vitro* systems that allow rapid, cost-effective and accurate predictions of *in vivo* immune responses would be enormous. A one-day workshop was convened by Division of AIDS, National Institutes of Health on August 4, 2010 to address the benefits and challenges of assessing HIV-1 vaccine responses in alternative ways. Consideration was given to the use of various *in vitro* model systems, human mucosal tissue explants and humanized mouse models as ways to predict immunogenicity and efficacy of HIV-1 vaccines early in the development process, and support decisions on whether a product may be worthy of moving into non-human primates or human trials. This report summarizes the outcome of the workshop.

1. Introduction

High throughput systems to evaluate the induction of primary immune responses to HIV-1 and HIV-1 vaccines have the potential to accelerate the discovery of new and relevant information and provide feedback for the next generation of clinical vaccines. These systems have the advantage to speed up the selection and facilitate development of promising candidate vaccines while decreasing the safety concerns and the costs associated with human clinical trials. Validation of these methodologies will require substantial evidence that the responses measured *in vitro* recapitulate responses elicited by *in vivo* vaccination or induced by acute HIV-1 infection.

The NIAID workshop was intended to review, discuss and obtain advice from expert participants (see Table 1) on the development and optimization of model systems to characterize and measure innate and adaptive immune responses to HIV-1 antigens, alone or in association with adjuvants, chemokines, cytokines or other immune-regulatory molecules. The workshop focused on the evaluation of prophylactic vaccines and approaches that employ human cells *in vitro*, *ex vivo* tissue and *in vivo* (humanized mouse). One part of the meeting was devoted to discussing approaches already underway that support the feasibility of efficiently optimizing the magnitude and functionality of the immune responses evoked *in vitro* by various antigens. Another part of the meeting was dedicated to evaluate the feasibility to test and compare vaccine candidates in human mucosal tissue explants. A final session was focused on the suitability of humanized mouse (hu-mouse) models to recapitulate the immune responses to HIV-1 infection and HIV-1 vaccines in humans. The goals of the workshop were to: (1) establish a plan to test, compare and optimize the various protocols currently in use by several teams across different laboratories with an outcome to develop optimal quantitative, high throughput and biologically relevant reproducible assays able to evaluate immune responses to HIV vaccines; (2) plan for parallel *in vitro*/*in vivo* studies to establish the predictive value of "*in vitro*" assays. Ultimately, *in vitro* studies on human cells and tissues may offer the opportunity to expedite the evaluation and comparison of different vaccine platforms in their effect on different arms of the immune system.

2. Challenges to *in vitro* priming

Although there was a consensus that current *in vitro* systems reflect enough of the *in vivo* response to make them useful models, they do not accurately reflect *in vivo* immunologic responses to prophylactic HIV-1 vaccines (see Table 2). Dr. Charles Rinaldo in the introductory lecture outlined some of the intrinsic limitations of *in vitro* systems which include the loss of the tissue architecture to appropriately replicate normal physiology and the effect that cell separation may have on their internal regulation and exocrine functions. From the practical standpoint the limited number of cells available and their relative short viability in culture constitute important shortcomings. *In vitro* systems face numerous other challenges that are inherent to the number and complexity of factors contributing to the immune responses following infection or vaccination. They include the nature and dose of the antigen, the kinetics of expression, and the route by which they would be administered. The choice of antigen presenting cells (APCs) employed *in vitro* to prime an effective adaptive immune response is also crucial, keeping in mind that cells like dendritic cells (DCs) have a key role in orchestrating the primary immune response. Indeed each APCs

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Table 1
Speakers to the "*In vitro* systems to characterize the immune response to HIV-1 and HIV-1 vaccine candidates", Workshop, August 4, 2010.

Speakers	Affiliations
Boggiano, Cesar	IAVI
Caskey, Marina	The Rockefeller University
Denton, Paul	University of North Carolina at Chapel Hill
Drake, Don	VaxDesign Corporation
Frahm, Nicole	Fred Hutchinson Cancer Research Center
Golding, Hana	US Food and Drug Administration
Koup, Richard	National Institutes of Health
Le Gall, Sylvie	Massachusetts General Hospital
Love, Christopher	Massachusetts Institute of Technology
Lubitz, Annika	ProBioGen AG, Belgium
Margolis, Leonid	National Institutes of Health
Moody, Tony	Duke Human Vaccine Institute
Palucka, Anna Karolina	Baylor Institute for Immunology Research
Pattijn, Sofie	Algonomics, Belgium
Charles Rinaldo	University of Pittsburgh
Sallusto, Federica	Institute for Research in Biomedicine, Switzerland
Sekaly, Rafick	Vaccine and Gene Therapy Institute-Florida
Shankar, Premlata	Texas Tech University
Stone, Geoffrey	University of Miami
Tager, Andrew	Massachusetts General Hospital

population has a distinctive capacity of antigen processing and presentation and depending on their maturation status they may or may not generate an effective immune response [1,2]. Multiple subsets of DCs are also known to employ different set of non-clonal recognition receptors (also called pattern recognition receptors) and the nature and triggering of those receptors has a profound influence on the strength, duration, and quality of T and B cell responses after vaccination [2–6].

Another key challenge of model systems is how the vaccine is formulated; often antigens are conjugated with an adjuvant, co-stimulatory molecule or other immunomodulatory molecules. In addition, distinct delivery systems are employed to enhance or direct the immune response to the antigen. The nature of the adjuvant can determine the particular type of immune response, which may be skewed toward cytotoxic T cell responses, particular classes of T helper responses or specific antibody isotypes. To define the right formulation for *in vitro* experimentations and for the development of surrogate *in vitro* models is crucial since *in vivo* effective protection against different pathogens requires distinct types of immune responses. It may be necessary to define for each intended outcome the most appropriate *in vitro* systems to use for screening.

On the T cell front one of the main challenges of *in vitro* systems is dealing with the very low frequency of T cell precursors that may recognize the specific antigen. The generation and expansion of primary T cell immune responses from naïve precursors *in vitro* has been difficult due to their restricted number and their stringent activation and costimulatory requirements [7–9]. Another major hurdle is the present limitation to appropriately regulate *in vitro* the expected quality of the T cell responses. This limitation is due to the inherent plasticity of the naïve T cells, which can adopt alternate lineage potentials upon engagement by complex innate immune mechanisms, specialized microenvironments, infection or vaccination that cannot be reproduced in a culture dish [10,11].

On the B cell front, the most important challenge recognized was the inability to reconstruct *in vitro* the complex microenviron-

Table 2
Key advantages and challenges of model systems.

System	Advantages	Challenges
In vitro – General	• No safety concern • Cost-effective • High-throughput • Rapid • Multi-laboratory • Standardizable	• Limited number of cells available, short cell viability and expansion in culture • Loss of tissue architecture and organ physiology • Limited types of cells and differentiation stages
In vitro – Innate immunity	• MDCCs efficiently prime naive T cells • Priming is susceptible to modulation by *in vitro* added cytokines • Peripheral Tissue Equivalent is a high throughput construct that replicates many physiologic innate immune responses	• The choice of the antigen presenting cells employed for priming a specific immune response is critical • Inadequate knowledge of the early events following infection and vaccination • Inability to reproduce the route of immunization • Mucosal milieu of the initial viral–DC interaction may not be recapitulated
In vitro – Cellular immunity	• Library of amplified T cells allows for a high throughput screening to determine the frequency of human naïve T cells specific for an antigen starting from only 100 to 2000 T cells • Provided that a library of identified epitopes exists the multiplex tetramers assays allows identifying the presence of T cell precursors for a specific vaccine in a single human blood sample	• Very low frequency of naïve T cell precursors recognizing a specific antigen • Inability to regulate the expected quality of the T cell responses • T cell repertoire development *in vitro* may not recapitulate what occurs *in vivo* due to insufficient knowledge of DC Ag processing, maturation, and activation phenotypes that occur in complex living systems
In vitro – Humoral immunity	• Responses to some antigens have been detected • Human artificial lymph node has the potential to recreate in part the tissue-like environment present in the lymph node • Lymphoid Tissue Equivalent module has the potential to recreate in part the conditions present in the lymph node	• Inability to reconstruct the complex microenvironment of the germinal centers and plasma foci • Poor viability and expansion of the B cells
Human tissue explants	• The tissue cytoarchitecture is preserved • Tissue explants support HIV replication without exogenous stimulation or activation • The system recapitulates *in vivo* pattern of HIV-1 replication • Viral "movement" in the tissue can be followed in real time by confocal microscopy	• Tissues start to deteriorate after 3 weeks in culture • The system does not reflect the effects of *in vivo* systemic factors or cell trafficking dynamics • The system is not high-throughput
Hu-Mouse models	• Relatively accessible and low cost • Recapitulation of the natural human resistance and susceptibility to specific pathogens • Reproducibility of the route of infection and immunization • Allows analysis of immune responses in the mucosal tissues	• Not all hu-mouse models are equivalent • The choice of the model is fundamental when obtaining information that are most predictive of the human condition • B cell development has not been fully elucidated • The system is not high-throughput