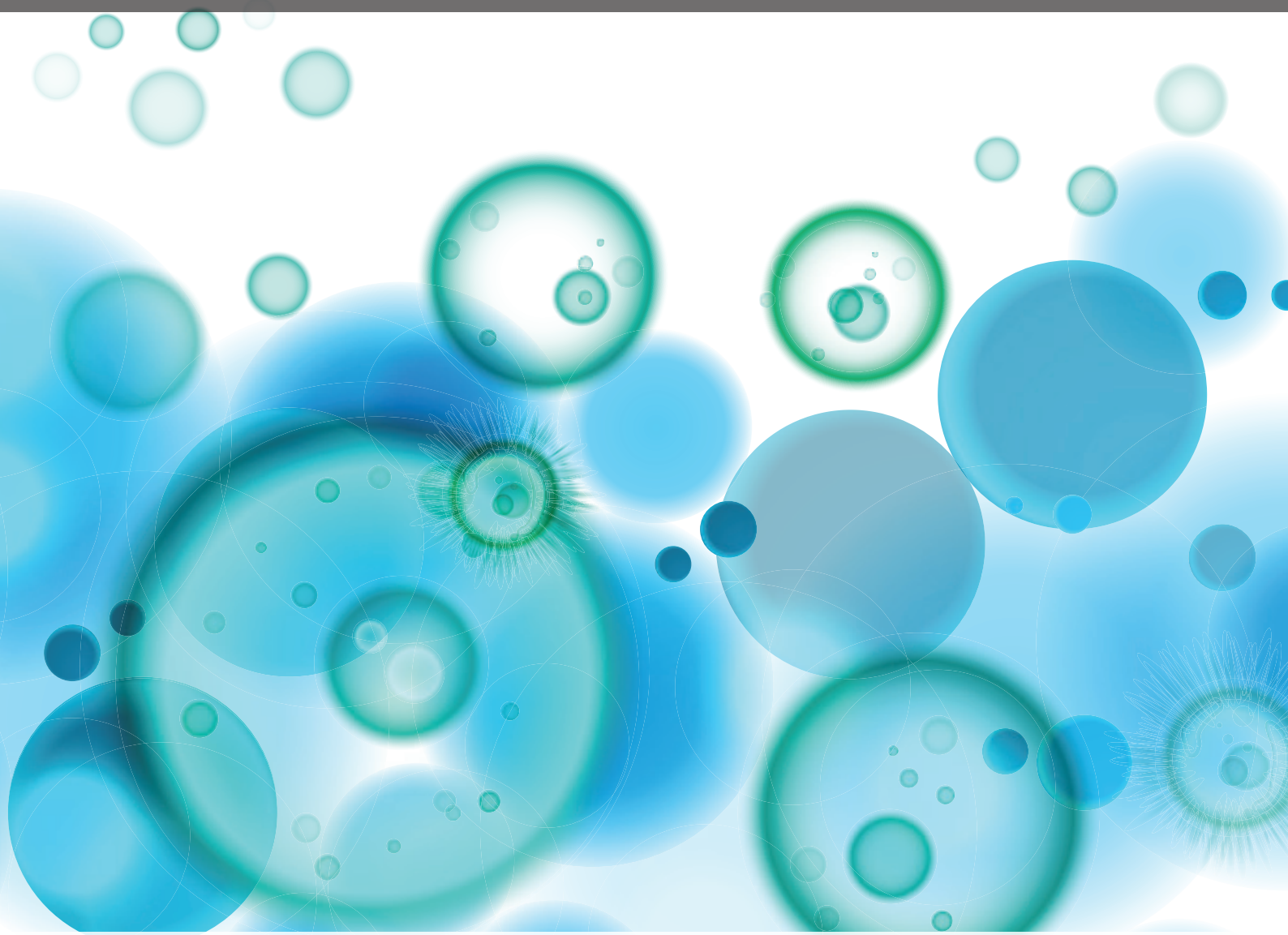
PARADIGM CHANGES ARE REQUIRED IN HIV VACCINE RESEARCH

EDITED BY: Marc H. V. Van Regenmortel
PUBLISHED IN: Frontiers in Immunology



frontiers Research Topics

frontiers

ISSN 1664-8714
ISBN 978-2-88919-727-9
DOI 10.3389/978-2-88919-727-9

About Frontiers

Frontiers is more than just an open-access publisher of scholarly articles: it is a pioneering approach to the world of academia, radically improving the way scholarly research is managed. The grand vision of Frontiers is a world where all people have an equal opportunity to seek, share and generate knowledge. Frontiers provides immediate and permanent online open access to all its publications, but this alone is not enough to realize our grand goals.

Frontiers Journal Series

The Frontiers Journal Series is a multi-tier and interdisciplinary set of open-access, online journals, promising a paradigm shift from the current review, selection and dissemination processes in academic publishing. All Frontiers journals are driven by researchers for researchers; therefore, they constitute a service to the scholarly community. At the same time, the Frontiers Journal Series operates on a revolutionary invention, the tiered publishing system, initially addressing specific communities of scholars, and gradually climbing up to broader public understanding, thus serving the interests of the lay society, too.

Dedication to quality

Each Frontiers article is a landmark of the highest quality, thanks to genuinely collaborative interactions between authors and review editors, who include some of the world's best academicians. Research must be certified by peers before entering a stream of knowledge that may eventually reach the public - and shape society; therefore, Frontiers only applies the most rigorous and unbiased reviews.
Frontiers revolutionizes research publishing by freely delivering the most outstanding research, evaluated with no bias from both the academic and social point of view.
By applying the most advanced information technologies, Frontiers is catapulting scholarly publishing into a new generation.

What are Frontiers Research Topics?

Frontiers Research Topics are very popular trademarks of the Frontiers Journals Series: they are collections of at least ten articles, all centered on a particular subject. With their unique mix of varied contributions from Original Research to Review Articles, Frontiers Research Topics unify the most influential researchers, the latest key findings and historical advances in a hot research area! Find out more on how to host your own Frontiers Research Topic or contribute to one as an author by contacting the Frontiers Editorial Office: **researchtopics@frontiersin.org**

PARADIGM CHANGES ARE REQUIRED IN HIV VACCINE RESEARCH

Topic Editor:
Marc H. V. Van Regenmortel, University of Strasbourg, France

In his 1962 book "The Structure of Scientific Revolutions", Thomas Kuhn famously argued that researchers in every field of scientific enquiry always operate under a set of presuppositions known as paradigms that are rarely explicitly stated.

In the field of HIV vaccine research, several prevailing paradigms led scientists for many years to pursue unfruitful lines of investigations that impeded significant progress. The uncritical acceptance of reigning paradigms makes scientists reluctant to abandon their mistaken assumptions even when they obtain results that are not consistent with the paradigms.

The following five paradigms which disregard the degeneracy of the immune system were particularly harmful.

1) There is a primary and intrinsic epitope specific for each B cell receptor and for the corresponding monoclonal antibody
   In reality, there is no single, intrinsic or "real" epitope for any antibody but only a diverse group of potential ligands
2) Reactions with monoclonal antibodies are more specific than the combined reactivity of polyclonal antibodies.
   In reality, a polyclonal antiserum has greater specificity for a multiepitopic protein because different antibodies in the antiserum recognize separate epitopes on the same protein, giving rise to an additive specificity effect. By focusing vaccine design on single epitope-Mab pairs, the beneficial neutralizing synergy that occurs with polyclonal antibody responses is overlooked.
3) The HIV epitope identified by solving the crystallographic structure of a broadly neutralizing Mab – HIV Env complex should be able, when used as immunogen, to elicit antibodies endowed with the same neutralizing capacity as the Mab.
   Since every anti HIV bnMab is polyspecific, the single epitope identified in the complex is not necessarily the one that elicited the bnMab. Since hypermutated Mabs used in crystallographic studies differ from their germline-like receptor version present before somatic hypermutation, the identified epitope will not be an effective vaccine immunogen.

4) Effective vaccine immunogenicity can be predicted from the antigenic binding capacity of viral epitopes.
   Most fragments of a viral antigen can induce antibodies that react with the immunogen, but this is irrelevant for vaccination since these antibodies rarely recognize the cognate, intact antigen and even more rarely neutralize the infectivity of the viral pathogen that harbors the antigen.
5) The rational design of vaccine immunogens using reverse vaccinology is superior to the trial-and-error screening of vaccine candidates able to induce protective immunity.
   One epitope can be designed to increase its structural complementarity to one particular bnMab, but such antigen design is only masquerading as immunogen design because it is assumed that antigenic reactivity necessarily entails the immunogenic capacity to elicit neutralizing antibodies. When HIV Env epitopes, engineered to react with a bnMab are used to select from human donors rare memory B cells secreting bnAbs, this represents antigen design and not immunogen design.

The aim of this Research Topic is to replace previous misleading paradigms by novel ones that better fit our current understanding of immunological specificity and will help HIV vaccine development.

Citation: Van Regenmortel, M. H. V., ed. (2015). Paradigm Changes are Required in HIV Vaccine Research. Lausanne: Frontiers Media. doi: 10.3389/978-2-88919-727-9

Table of Contents

frontiers in Immunology

EDITORIAL
published: 23 June 2015
doi: 10.3389/fimmu.2015.00326

Editorial: Paradigm changes are required in HIV vaccine research

*Marc H. V. Van Regenmortel**

CNRS, UMR 7242 Biotechnologie et Signalisation Cellulaire, Institut de Recherche de l'Ecole de Biotechnologie de Strasbourg (IREBS), Ecole Supérieure de Biotechnologie Strasbourg (ESBS), University of Strasbourg, Illkirch, France

Keywords: antibody affinity maturation, paradigms in vaccine research, rational HIV-1 vaccine design, reverse vaccinology, vaccine immunogenicity

In his influential book "The Structure of Scientific Revolutions" published in 1962, Thomas Kuhn argued that researchers in every field of scientific enquiry are always guided by theoretical assumptions, presuppositions, and hypotheses that constitute the prevailing scientific paradigm under which they operate at any given time (1). When they operate within a shared paradigm, a scientific community working in a particular field will be committed to using certain strategies and experimental approaches that are accepted as being essential for trying to solve the problem at hand.

In many cases, the implicit hypotheses and presuppositions that give rise to a particular paradigm are not clearly stated and investigators may therefore sometimes not even be aware of them. One consequence of this is that when they obtain results that are not compatible with the theory and hypotheses underlying the paradigm, they may fail to appreciate that their guiding paradigm has in fact been refuted and should therefore be abandoned or revised. On the other hand, according to Kuhn, scientists actually do not abandon their hypotheses and paradigms as soon as contradictory data are obtained, since their main goal is not to try to confirm the validity of the underlying assumptions of their paradigms. Scientific communities tend to remain committed to their shared theoretical beliefs even when they obtain anomalous results that are not consistent with the paradigm, and they will invent new *ad hoc* hypotheses in an attempt to resolve apparent contradictions between theory and experimental observations (2). Unfortunately, when misleading paradigms are not discarded, this may encourage scientists to pursue unfruitful lines of investigations that could impede scientific progress.

In the field of HIV vaccine research, there is in fact evidence that in recent years a number of paradigms based on invalid assumptions had such a detrimental effect (3). This drawback can only be avoided if scientists keep in mind that if the paradigms they have adopted are based on erroneous assumptions, this could lead them to select inappropriate research strategies that are unlikely to succeed. Questioning the validity of paradigms is thus an important safeguard since it may reveal which invalid assumptions have led investigators astray in the past. Only when past mistakes are acknowledged can novel paradigms be introduced, which better fit our improved knowledge of HIV-1 immune responses, and are therefore more likely to help future vaccine development (4, 5).

The following unwarranted assumptions underlying some popular paradigms in HIV vaccine research may have contributed to our inability during the past 25 years to develop an effective HIV-1 vaccine.

Assumption No. 1: Vaccine Immunogenicity can be Predicted from Viral Antigenicity

Most fragments of a viral antigen are immunogenic and are able to induce antibodies that will react with the fragment. However, this type of immunogenicity is irrelevant for vaccination since these antibodies rarely recognize the cognate, intact antigen, and even more rarely neutralize the infectivity of the viral pathogen that harbors the antigen (6). A confusion between biological immunogenicity (the ability to induce antibodies in a host) and chemical antigenicity (the capacity of an antigen to bind antibodies) lies at the heart of the reverse vaccinology paradigm that has been pursued vigorously for

OPEN ACCESS

Edited by:
Kendall Arthur Smith, Weill Medical College of Cornell University, USA

Reviewed by:
Sarah Rowland-Jones, John Radcliffe Hospital, UK

***Correspondence:**
Marc H. V. Van Regenmortel
vanregen@unistra.fr

Specialty section:
This article was submitted to HIV and AIDS, a section of the journal Frontiers in Immunology

Received: *10 March 2015*
Accepted: *10 June 2015*
Published: *23 June 2015*

Citation:
Van Regenmortel MHV (2015) Editorial: Paradigm changes are required in HIV vaccine research. Front. Immunol. 6:326. doi: 10.3389/fimmu.2015.00326

more than 10 years. According to this paradigm, HIV-1 epitopes identified by X-ray crystallographic analysis of complexes of HIV Env bound to affinity-matured neutralizing monoclonal antibodies (nMabs) were expected to be also effective vaccine immunogens able to induce a protective immune response. However, an epitope that binds to a nMab will not necessarily be able to induce the same type of neutralizing antibody in an immunized host. As discussed at length elsewhere (7), investigators who claim they are designing a vaccine immunogen are only improving the binding reactivity (i.e., the antigenicity) of a single epitope–paratope pair and are not actually designing a vaccine immunogen able to generate protective antibodies. Immunogenicity does depend on numerous factors that exist only in the context of the host immune system, and these are independent of the binding properties of the viral antigen used for immunization (8).

Assumption No. 2: There is a Primary and Intrinsic Epitope Specific for Each B Cell Receptor and its Corresponding Antibody

In reality, there is no single intrinsic epitope for any antibody molecule but only a diverse group of potential ligands able to bind to it with various degrees of fit (3). Vaccinologists have been slow to accept that antibodies are not monospecific for a single epitope and that the degeneracy of the immune system always makes antibodies polyspecific for numerous related or unrelated epitopes present in different antigens (9–11). Once it is accepted that the epitope identified by X-ray crystallography of an HIV-1 Env-nMab complex is only one of the many epitopes that could be accommodated by that antibody, there is no justification for assuming that this particular HIV-1 epitope must correspond to the immunogen that elicited the nMab and should therefore be considered as a possible candidate vaccine.

Assumption No. 3: HIV-1 Epitopes Recognized by Mature nMabs Isolated from HIV-1 Infected Individuals after a Lengthy Process of Antibody Affinity Maturation will be able to Induce a Protective Immune Response in Naive Individuals

It has been established that the initial HIV-1 immunogen that triggers the affinity maturation process leading to mature neutralizing antibodies usually recognizes a BCR germline version that differs considerably from the BCRs corresponding to mature antibodies (12). The very extensive affinity maturation required to obtain neutralizing HIV-1 antibodies is one of the main reasons why the structure-based reverse vaccinology approach did not succeed in developing an effective HIV-1 vaccine (3). A new paradigm based on the germline/maturation hypothesis was subsequently developed, which assumed that it may be possible to discover effective HIV-1 vaccine immunogens by analyzing putative germline antibody intermediates of known HIV-1 nMabs (13). It is not clear at present whether the unraveling of large numbers of different antibody maturation pathways will allow the identification of HIV-1 vaccine immunogens suitable for vaccinating large human populations.

Assumption No. 4: The so-called Rational Design of HIV-1 Immunogens is More Effective than the Classical Trial-and-Error Screening of Immunogens used in the Past for Developing Successful Vaccines

Many authors claim that so-called "rational design" offers the best prospects for developing an HIV-1 vaccine. Design means the deliberate conceiving of a novel object or process by an intelligent being, while the term "rational" is mostly used to indicate that the designer makes use of available molecular data (8, 14). When they discuss the rational design of HIV-1 vaccines, these authors refer only to studies that try to improve the structural complementarity between one epitope and one particular Mab, which means they actually discuss antigen design and not immunogen design (15, 16). Designing vaccine immunogenicity actually means modifying an antigen, so that it becomes an immunogen capable of inducing a protective immune response and this requires investigating empirically the numerous factors, which in an immunized host determine the formation of neutralizing antibodies. Unfortunately, we know very little about the immunological mechanisms in the host that produce neutralizing rather than non-neutralizing antibodies and our ignorance is what prevents us from deliberately "designing" vaccine immunogens of predetermined, known efficacy. Rationally designing an antigen so that it better fits a single Mab is certainly possible but improving an immunogen so that it is better able to elicit protective antibodies can only be achieved by trial-and-error experimentation with various immunogens and not by design (8, 17). It is currently fashionable to denigrate empirical approaches in vaccine research (18), as if time-honored empiricism and trial-and-error experimentation were not an entirely rational enterprise compatible with high quality science (3, 7, 14). Advocating rational design instead of stressing the need for more empirical research may even be counterproductive since it is likely to make it more difficult for investigators to undertake the small exploratory trial-and-error trials that are needed to move the field forward (5). Although epitope mapping with nMabs may indicate which regions of a virus surface should be targeted by a vaccine, such mapping on its own will not make it possible to discover effective vaccine immunogens if no immunogenicity trials are performed.

Assumption No. 5: Reactions of Viral Antigens with Mabs are More Specific than the Combined Reactivity of Polyclonal Antibodies Found in an Antiserum and are able to Inform Vaccine Design

The introduction of Mabs revolutionized our ability to dissect immune responses to proteins but it also introduced a bias in the analysis of antigens by encouraging investigators to focus on

artificial boundaries between overlapping epitopes and to concentrate on single, discrete epitopes as potential vaccine immunogens. The emphasis on single epitopes recognized by Mabs for developing vaccines tends to obscure the beneficial effect of the neutralizing synergy achievable with polyclonal responses (19).

The use of Mabs for characterizing epitopes also introduced another bias that occurs because the apparent specificity of a Mab very much depends on the selection process that was used to obtain it. When a nMab, for instance, binds to a short peptide region of the membrane proximate external region (MPER) of HIV-1 gp41, this may simply reflect the fact that the Mab was selected for its ability to bind to that peptide. Since all antibodies are polyspecific, the Mab may actually recognize better a more complex or transient epitope of gp41 that might have been the immunogen that elicited the antibody. However, if it is assumed that the Mab was induced by the linear MPER peptide region because it reacts with it, searching for an effective vaccine immunogen in gp41 may be compromised.

It is often believed that a Mab that binds to a given antigen is a more specific reagent than a polyclonal antiserum raised against that antigen. In reality, an antiserum has a greater collective specificity for a multiepitopic antigen due to the additive specificity effect that arises from the presence in the antiserum of antibodies directed to several different epitopes of the antigen (20).

It is sometimes suggested that the isolation of additional broadly neutralizing Mabs will facilitate the future rational design of an HIV-1 vaccine. Why this should be the case is not made clear since these Mabs only have the potential to perhaps 1 day become useful reagents for *passive* immunotherapy. However, their isolation does not improve our ability to discover which vaccine immunogens will elicit neutralizing antibodies by means of *active* immunization (14). Once again, confounding antigenicity and immunogenicity is giving rise to unrealistic expectations.

The aim of this Research Topic is to evaluate the shortcomings of some of the paradigms that guided HIV vaccine research in the past as well as to stimulate the search for novel paradigms that better fit our current understanding of immunological specificity and could be more helpful in guiding the future search for an effective HIV-1 vaccine.

References

1. Kuhn TS. *The Structure of Scientific Revolutions*. Chicago: University of Chicago Press (1962).
2. Bird A. Thomas kuhn. Fall 2013 ed. In: Zalta EN, editor. *The Stanford Encyclopedia of Philosophy*. (2013). Available from: http://plato.stanford.edu/entries/thomas-kuhn/
3. Van Regenmortel MHV. An outdated notion of antibody specificity is one of the major detrimental assumptions of the structure-based reverse vaccinology paradigm which prevented it from helping to develop an effective HIV-1 vaccine. *Front Immunol* (2014) **5**:593. doi:10.3389/fimmu.2014.00593
4. Esparza J. What has 30 years of HIV research taught us? *Vaccines* (2013) **1**:513–26. doi:10.3390/vaccines1040513
5. Van Regenmortel MHV, Andrieu J-M, Dimitrov DS, Ensoli B, Hioe CE, Moog C, et al. Paradigm changes and the future of HIV vaccine research: a summary of a workshop held in Baltimore on 20 November 2013. *J AIDS Clin Res* (2014) **5**:281. doi:10.4172/2155-6113.1000281
6. Van Regenmortel MHV. Immunoinformatics may lead to a reappraisal of the nature of B cell epitopes and of the feasibility of synthetic peptide vaccines. *J Mol Recognit* (2006) **19**:183–7. doi:10.1002/jmr.768
7. Van Regenmortel MHV. Why does the molecular structure of broadly neutralizing monoclonal antibodies isolated from individuals infected with HIV-1 not inform the rational design of an HIV-1 vaccine? *Aims Public Health* (2015) **2**:183. doi:10.3934/publichealth.2015.2.183
8. Van Regenmortel MHV. Requirements for empirical immunogenicity trials, rather than structure-based design, for developing an effective HIV vaccine. *Arch Virol* (2012) **157**:1–20. doi:10.1007/s00705-011-1145-2
9. Sperling R, Francus T, Siskind GW. Degeneracy of antibody specificity. *J Immunol* (1983) **131**:882–5.
10. Notkins AL. Polyreactivity of antibody molecules. *Trends Immunol* (2004) **25**:174–9. doi:10.1016/j.it.2004.02.004
11. Eisen HN, Chakraborty AK. Evolving concepts of specificity in immune reactions. *Proc Natl Acad Sci U S A* (2010) **107**:22373–80. doi:10.1073/pnas.1012051108
12. Dimitrov DS. Therapeutic antibodies, vaccines and antibodyomes. *MAbs* (2010) **2**:347–56. doi:10.4161/mabs.2.3.11779
13. Prabakaran P, Chen W, Dimitrov DS. The antibody germline/maturation hypothesis, elicitation of broadly neutralizing antibodies against HIV-1 and cord blood IgM repertoires. *Front Immunol* (2014) **5**:398. doi:10.3389/fimmu.2014.00398
14. Van Regenmortel MHV. Basic research in HIV vaccinology is hampered by reductionist thinking. *Front Immunol* (2012) **3**:194. doi:10.3389/fimmu.2012.00194
15. Douek DC, Kwong PD, Nabel GJ. The rational design of an AIDS vaccine. *Cell* (2006) **124**:677–81. doi:10.1016/j.cell.2006.02.005
16. Nabel GJ, Kwong PD, Mascola JR. Progress in the rational design of an AIDS vaccine. *Philos Trans R Soc Lond B Biol Sci* (2011) **366**:2759–65. doi:10.1098/rstb.2011.0096
17. Van Regenmortel MHV. The rational design of biological complexity: a deceptive metaphor. *Proteomics* (2007) **6**:965–75. doi:10.1002/pmic.200600407
18. Karlsson Hedestam GB, Fouchier RA, Phogat S, Burton DR, Sodroski J, Wyatt RT. The challenges of eliciting neutralizing antibodies to HIV-1 and to influenza virus. *Nat Rev Microbiol* (2008) **6**:143–55. doi:10.1038/nrmicro1819
19. Van Regenmortel MHV. Limitations to the structure-based design of HIV-1 vaccine immunogens. *J Mol Recognit* (2011) **24**:741–53. doi:10.1002/jmr.1116
20. Van Regenmortel MHV. Specificity, polyspecificity and heterospecificity of antibody-antigen recognition. *J Mol Recognit* (2014) **27**:627–39. doi:10.1002/jmr.2394

Conflict of Interest Statement: The author declares that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in
IMMUNOLOGY

REVIEW ARTICLE
published: 18 March 2015
doi: 10.3389/fimmu.2015.00124

A new scientific paradigm may be needed to finally develop an HIV vaccine

José Esparza*

Institute of Human Virology, University of Maryland School of Medicine, Baltimore, MD, USA

Edited by:
Hanneke Schuitemaker, Crucell Holland BV, Netherlands

Reviewed by:
Ann Jones Hessell, Oregon Health & Science University, USA
Francisco Veas, Institut de recherche pour le Développement (IRD), France

****Correspondence:***
José Esparza, 11990 Market Street, Unit #212, Reston, VA 20190, USA
e-mail: jose.esparza5@live.com

The bulk of current HIV vaccine research is conducted within the infectious disease paradigm that has been very successful in developing vaccines against many other viral diseases. Different HIV vaccine concepts, based on the induction of neutralizing antibodies and/or cell mediated immunity, have been developed and clinically tested over the last 30 years, resulting in a few small successes and many disappointments. As new scientific knowledge is obtained, HIV vaccine concepts are constantly modified with the hope that the newly introduced tweaks (or paradigm drifts) will provide the solution to one of the most difficult challenges that modern biomedical research is confronting. Efficacy trials have been critical in guiding HIV vaccine development. However, from the five phase III efficacy trials conducted to date, only one (RV144) resulted in modest efficacy. The results from RV144 were surprising in many ways, including the identified putative correlates of protection (or risk), which did not include neutralizing antibodies or cytotoxic T-cells. The solution to the HIV vaccine challenge may very well come from approaches based on the current paradigm. However, at the same time, out-of-the-paradigm ideas should be systematically explored to complement the current efforts. New mechanisms are needed to identify and support the innovative research that will hopefully accelerate the development of an urgently needed HIV vaccine.

Keywords: HIV-1 vaccines, paradigm shift, non-neutralizing antibodies, vaccine efficacy trials, innovation

KUHN'S VIEWS OF NORMAL SCIENCE AND PARADIGM CHANGE

The bulk of the current scientific activity, at least the one that it is publically funded, is generally conducted within what is known as normal science.

The term "normal science" was proposed by Thomas Kuhn in his 1962 book entitled "*The Structure of Scientific Revolutions*" (1). In his book, Kuhn describes normal science as the "research that it is firmly based upon one or more past scientific achievements, achievements that some particular scientific community acknowledges for a time as supplying the foundation for its further practice." Those past achievements are generally well-documented in the scientific literature, and serve "to define the legitimate problems and methods of a research field for succeeding generations of practitioners." In addition, Kuhn coined the term "paradigm" to define the achievements that were "sufficiently unprecedented to attract an enduring group of adherents" and at the same time, "sufficiently open ended to leave all sorts of problems for the redefined group of practitioners to resolve."

Normal science produces much useful information, but Kuhn proposed that normal science results in little major novelties and it could be argued that it is mostly gap-filling science. Unexpected results that cannot be explained by the current paradigm are frequently ignored or dismissed. When the paradigm is incapable of producing a solution, it enters into a crisis, this leading to a "paradigm shift" which changes the basic assumptions within the ruling theory of science. Then, under the light of a more satisfactory paradigm, some of the previously dismissed observations are acknowledged and understood, and new avenues are opened to conduct new research and to finally solve the problem.

CURRENT HIV VACCINE PARADIGM AND DRIFTS THAT HAVE OCCURRED OVER THE PAST 30 YEARS

Although it is not straightforward to formally define the current HIV vaccine development paradigm, it is not very different from the one that started in the late 1800s, when the germ theory of disease was formulated (2). In summary, the infectious disease vaccine paradigm proposes that different germs cause different diseases, and that the protective adaptive immune responses resulting from the disease can also be stimulated by weakened (attenuated) or killed (inactivated) microorganisms in the form of vaccines. This paradigm has been extremely successful in defining the etiology of major killer diseases of mankind. It has also resulted in the development of vaccines and drugs against many infectious diseases.

However, the infectious disease paradigm, like many other satisfactory paradigms, has gone through minor changes (or paradigm drifts). One change occurred when viruses were described as being structurally different from bacteria, although that did not prevent Louis Pasteur from empirically developing a rabies vaccine, even before viruses were formally discovered (3). In the 1970s retroviruses were widely acknowledged as the cause of cancer and leukemia in animals, but there was strong reluctance to accept the idea that retroviruses could cause infections in humans. When Robert Gallo and collaborators proposed in 1979 that a new retrovirus, the human T-cell lymphoma virus 1 (HTLV-1), causes

adult T-cell leukemia (ATL), he found resistance to have the paper accepted for publication, because it represented a departure from the commonly accepted science (4, 5). Fortunately, the common science that was conducted in the 1970s under the "War on Cancer" Program (6) developed the basic tools of retrovirology that also allowed the rapid isolation and characterization of the human immunodeficiency virus (HIV) soon after AIDS was recognized as a new disease in 1981. This is a remarkable example of how research supported to solve a given problem (cancer in this case) can yield unanticipated results in another field (AIDS). What is important is to always keep an open mind.

The decades of the 60s and 70s witnessed the birth of modern molecular biology (7) and rapid progress was made in the understanding of the structure–function relationships in animal viruses. German virologists made many of the pioneering discoveries (8), especially the school of Werner Schäfer (1912–2000) at the Max-Planck-Institut fúr Virusforschung in Tubingen. Studying fowl plague virus (FPV) and other influenza A viruses, Schäfer and collaborators established that the haemaglutinin (HA) serves as a ligand during attachment of the virus cellular receptor and that it was also the immunogen which induces the production of protective neutralizing antibodies in the infected host. Schäfer went on to say in 1963 that "the finding that the immunizing capacity of the fowl plague virus resides in its hemagglutinin shell material led to the proposal to use, instead of inactivated total virus the isolated hemagglutinin of influenza and possibly other myxoviruses as vaccines" (9).

Although we cannot consider this proposed subunit approach as a completely new paradigm, it was obviously a major departure (a paradigm drift) from how viral vaccines were successfully developed until then, based on inactivated or attenuated viruses. It is interesting that two American scientists who were to occupy important roles in the early HIV vaccine effort in the United States, Dani Bolognesi (a virologist from Duke University who became the co-chair of the US government's AIDS Vaccine Working Group) and Peter Fischinger (who became the first AIDS Coordinator from the US Department of Health and Human Services), had worked with Werner Schäfer in Tubingen, studying the structure–function relationship in animal retroviruses (10). They quickly translated those concepts to the search for an HIV vaccine (11, 12), confirming that the envelope glycoprotein of HIV was sufficient to induce the production of neutralizing antibodies, as it has been shown earlier with the myxoviruses (13).

Since HIV is a very dangerous pathogen, the prospect of using a subunit vaccine was daunting, both in relation to manufacturing issues and because of potential risks to the vaccinated individuals. The response to this challenge was provided by the emerging science of genetic engineering and recombinant DNA technology, which provided the possibility of manufacturing large amounts of viral proteins without actually growing the virus. The most relevant precedent at that time was that of the vaccine against the hepatitis B virus. A highly effective plasma-derived hepatitis B vaccine had been licensed by the US Food and Drug Administration (FDA) in 1981. However, concerns were rapidly raised because the source of the vaccine immunogen was the plasma of individuals who could also be carrying the AIDS virus. The solution came when the surface antigen of the hepatitis B virus was successfully cloned and expressed in yeast, allowing for the manufacturing of a recombinant hepatitis B vaccine, which was licensed in 1986 (14, 15).

Thus, the first wave of HIV vaccine development, based on genetically engineered subunit envelope vaccines (16), was based on several past achievements of normal science: (a) the demonstration that the envelope glycoproteins of the virus are sufficient to induce neutralizing antibodies; (b) the ability to manufacture large amounts of these proteins by genetic engineering techniques; and (c) the successful proof of concept provided by the recombinant hepatitis B vaccine.

This initial vaccine effort also benefited from rapid advances in the molecular biology of HIV that occurred within 5 years after its discovery, including the identification of the major structural proteins of the virus, the cloning and sequencing of the HIV genome, early information on the genetic variability of different virus strains, the description of neutralizing antibodies, and the development of the first non-human primate models (17).

Although in the late 1980s nobody knew for sure how long it would take to develop an HIV vaccine, it is also fair to say that the field was generally optimistic. The prediction was made, and repeated many times since then, that an HIV vaccine would be available within the next 10 years. However, no one knew at that time that HIV/AIDS was much more complex than any other viral disease for which vaccines had been successfully developed (18–21). Nevertheless, phase I clinical trials of HIV envelope vaccines started in the United States in 1988, thus beginning a long history of small successes and big disappointments.

In previous articles, I have discussed in detail the three major approaches that have been explored over the last 30 years in trying to develop an HIV vaccine (17, 22). Although I have described those three waves of vaccine approaches and clinical trials as based on three different paradigms, in fact they only represented allowable tweaks (or drifts) within the overarching infectious disease paradigm. The first wave started around 1984 and it was based on the concept that neutralizing antibodies would be sufficient to confer protection against HIV infection. This led to the development of numerous recombinant envelope-based candidate vaccines that were tested in clinical trials. This first wave came to an end in 2003, with the negative results from two efficacy trials designed to evaluate the protective efficacy of the gp120 vaccines from VaxGen (23, 24). The second wave began with the recognition in the early 2000s of the critical importance of CD8+ T-cell responses in the control of HIV infection, and this led to the development and refinement of live recombinant viral vectors, especially poxvirus and adenovirus vectors, as well as DNA vaccines. This period was formally concluded in 2007 with the unexpected lack of efficacy in the STEP trial, which evaluated a cell-mediated immunity vaccine based on an adenovirus type 5 (Ad5) vector (25). The third wave may have started in 2009 with the modest efficacy obtained in the RV144 trial conducted in Thailand, to evaluate a prime boost combination of an ALVAC vector followed by an envelope glycoprotein (26). This wave, that hopefully will take us to the development of an effective vaccine, should learn from past failures and systematically explore different alternatives, including novel concepts that do not fall within the current paradigm.

However, it is fair to say that the first two waves just described (antibodies and cell-mediated immunity) have not completely ended. Instead, they are constantly revisited when new knowledge is obtained, and numerous adjustments have been made, representing allowable drifts within the current paradigm, with the hope that those changes would eventually led to the solution of the problem.

For example, one of the first conceptual drifts in the antibody approach occurred around 1994 with the realization that laboratory-adapted strains of HIV behave immunologically differently from the primary/clinical isolates (27). That paradigm drift led to the design of more sophisticated envelope immunogens, such as those based on founder/transmitted viruses (28) or those using envelope trimers rather than the gp120 monomers initially used to develop HIV candidate vaccines (29, 30). It is also relevant to mention here that the hope of designing epitope-based HIV vaccines started as early as 1989, when the V3 loop of gp120 was thought to be, and even referred to, as the Principal Neutralization Domain (PND) (31), a vaccine concept that even progressed to phase I clinical trials (32). That early peptide-based HIV vaccine approach was eventually abandoned when it was realized that complex conformational epitopes are important for the induction of neutralizing antibodies (33).

However, a major driver of the more recent approaches in the antibody field has been the need to deal with the immunological variability of HIV strains and clades. The discovery that broadly neutralizing antibodies recognize defined epitopes in the envelope trimer, which has led to a renewed effort to develop epitope-based vaccines guided by structural biology (34, 35), a reductionist approach that claims to have achieved proof of concept with the respiratory syncytial virus (36), but which has been strongly criticized by others (37, 38). The difficulties in designing an epitope-based vaccine capable of eliciting broadly neutralizing antibody responses is compounded by the extensive affinity maturation process that anti-HIV neutralizing antibodies undergo before acquiring the broadly neutralizing characteristics (39). This challenge is being addressed by the use of sequential immunization with different HIV envelope immunogens designed to guide the evolution of the antibody, triggering the selection and expansion of germline precursor and intermediate memory B cells to recapitulate B cell ontogenies associated with the maturation of a broadly neutralizing antibody response (40, 41), a concept that had been proposed several years before (42). Others, perhaps more practical approaches, have been proposed to develop a globally relevant HIV vaccine capable of protecting against a variety of strains and clades (43), including the use of mosaic immunogens (29, 44).

On the other hand, most of the paradigm drifts in the field of cell-mediated HIV vaccines have focused on the use of different vectors, or prime-boost approaches, with the object of eliciting stronger and more functional CD8+ responses to selected HIV proteins (45–47). Perhaps, the most significant paradigm drift in the cell-mediated immunity field is represented by the report that the early control elicited by a simian immunodeficiency virus (SIV) protein-expressing rhesus cytomegalovirus (RhCMV) vectors (48) was due to SIV-specific CD8+ effector memory T cells that recognize unusual, diverse, and highly promiscuous epitopes, including dominant responses to epitopes restricted by class II major histocompatibility complex (MHC) molecules (49–51).

EFFICACY TRIALS HAVE BEEN INSTRUMENTAL IN DRIFTING PARADIGMS AND ADVANCING VACCINE RESEARCH

In the absence of predictive animal models, or of known immune correlates of protection, the only approach to assess the protective efficacy of any HIV vaccine concept is by conducting large scale efficacy trials of the candidate vaccines considered to be the most promising. However, what is considered to be the "most promising" candidate vaccines usually is in the eyes of the beholder. Since efficacy trials are complex and expensive, it is widely recognized that a decision to proceed to phase III trials needs to be based on the best science available, and this responsibility should not be taken lightly. On the other hand, the urgent public health need of an HIV vaccine should be considered in order not to delay those important decisions (52).

A case in point is the RV144 efficacy trial conducted in Thailand between 2003 and 2009. The trial was strongly opposed by a group of respected scientists, who were not convinced of its scientific merits (53). Anyway, the trial went ahead and it was conducted almost totally ignored by the scientific community. When the announcement was made in 2009 that the RV144 trial showed modest efficacy (26), it came as a surprise and the results were initially received with skepticism. After all, the results contradicted what was commonly accepted at that time, namely that the most likely protection that a vaccine could provide was against virus load and not against virus acquisition. In addition, the identified immune correlates of protection were not the usual suspects (neutralizing antibodies or CD8+ T cells). Instead, a still to be better defined non-neutralizing antibody response to the V1–V2 loops of gp120 was found to be the strongest correlate of protection (54). Subsequent laboratory studies have strengthened the conviction that the protective efficacy observed in RV144 is true, and have identified IgG3 antibodies as an additional potential correlate of protection (55).

The results from the RV144 trial have stimulated new research on antibody functions other than neutralization, such as antibody-dependent cell-mediated cytotoxicity (ADCC) (56, 57), which is slowly becoming part of the accepted normal science in HIV vaccine research, side by side with the better understood neutralizing antibodies. The newly acquired respectability of ADCC should help understanding the earlier results reported by Robert Gallos's team in 2007 using subunit immunogens designed to raise humoral responses against CD4-induced (CD4i) epitopes (21, 58, 59). Even earlier, in 2005, ADCC was reported as an immune correlate of protection against SIV in non-human primate protection (60). However, the results from those experiments were received with skepticism or even indifference, because at that time our minds were not prepared to think outside of the box regarding antibody functions. The classical neutralizing antibodies are probably the most important mechanism of protection against HIV, as is the case with most viral vaccines, but the potential role of ADCC and of other antibody functions should not be dismissed *a priori* (61, 62).

From the five efficacy trials of HIV vaccines that have been completed in the last 10 years, only RV144 showed efficacy, albeit

modest (17, 23–26, 63). To build on the success of the RV144 trial, a group of organizations established the Pox-Protein Public-Private Partnership (P5) to evaluate potentially improved pox-protein vaccines to determine if they might provide significant public health benefit, with follow-up clinical studies using improved vaccine regimens being planned in southern Africa and Thailand (64). A very important objective of the P5 is to validate the hypothesis that in the RV144 trial, antibodies directed against the V1–V2 loops may have contributed to protection against HIV-1 infection, whereas high levels of envelope-specific IgA antibodies may have mitigated the effects of protective antibodies (54). What is now critical is to develop strict and credible go/no-go criteria to determine if the potentially improved vaccines should move from phase I clinical trials to large scale efficacy evaluation, including the ability to test the hypotheses generated by the RV144 trial. In making that decision, it is important to keep in mind that the RV144 trial was conducted in Thailand in a population with relatively low risk behavior and an annual HIV incidence of approximately 0.2% (65, 66), and that the proposed P5 trials are planned to be conducted in populations with annual HIV incidences in the order of 3–9%. One could argue that the vaccines to be tested by the P5 collaborators should be proportionally improved, considering the stronger force of infection in the proposed new testing population.

Since phase III efficacy trials are large and expensive, every effort should be made to obtain pre-clinical and early clinical evidence to justify such a decision. Although non-human primate protection experiments are instructive, and a positive result would add confidence to a decision to move to efficacy evaluation, they are not necessarily considered as predictive of results in humans. An alternative, or rather complementary approach to select candidate vaccines for further evaluation, has been proposed by testing candidate vaccines in a handful of human volunteers whose immune system is intensively interrogated in the search for clues that may suggest the induction of protective immunity. These small trials, referred by some as "Experimental Medicine" (EM) trials, could be very valuable for vaccines for which we have known immune correlates of protection (67), but they present a challenge for HIV. However, we can imagine that envelope immunogens designed to induce broadly neutralizing antibodies, including approaches that guide their maturation, could be tested in EM trials (40). Likewise, human CMV vectors could be tested in EM trials to assess if they recapitulate in humans the potentially protective immune responses that have been identified in rhesus monkeys (49).

Perhaps, the identification of a single protective epitope or of a single immune correlate of protection is an illusion derived from our desire to reduce complex biological phenomena to simple explanations and approaches. Rather than thinking about just one individual immune correlate, we should seriously consider that protection is associated with a more complex immunological signature of immune responses. Fourteen years ago, Neil Nathanson and Bonnie Mathieson, from the Office of AIDS Research of the US National Institutes of Health, speculated that a "possible explanation for the inconsistent conclusions from studies of SIV and SHIV models is that protection does not correlate with any single immune response but is conferred by a barrier created by the sum of several immune defenses" (68). In fact, the coordinated activity of multiple antibody functions has been recently suggested as the mechanism of protection in a proposed globally relevant HIV-1 mosaic vaccine (44).

CAN WE IDENTIFY INNOVATION WHEN WE SEE IT?

There is no doubt that many of the paradigm drifts introduced over the years to the original antibody and cell-mediated immunity concepts have been very innovative, although they have not represented significant shifts of the prevailing paradigm. Scientists have stubbornly pursued their belief that the current paradigm, once it is appropriately modified, will provide the solution. Resilience is important for the progress of science. However, and paraphrasing the immunologist and Nobel Prize winner Peter Medawar, "the intensity of the conviction that a hypothesis is true has no bearing on whether it is true or not" (69). Nevertheless, Medawar emphasized that the strength of the scientists' convictions is important if only because that conviction provides the necessary incentive to conduct the research to find out if the hypothesis is correct.

The scientific community is generally open to accept innovation when it falls within the accepted paradigms of normal science. However, the same community is often reluctant to accept ideas that fall outside of the paradigm. The possible reasons for this attitude are that most of those out-of-the paradigm ideas are: (a) sometimes proposed without much preliminary data, (b) not supported by a community of peers and, (c) in many cases, cannot stand up to critical scrutiny or to experimental verification.

The question that we are now trying to answer is if after 30 years of intense work, the current paradigm to develop an HIV vaccine is entering into a crisis, thus requiring a paradigm shift. Is it sufficient to go back to the same drawing board every time we experience a major failure? (70), or should we explore more systematically completely new avenues of research? Perhaps the nature of HIV and AIDS, which significantly differ from other viral diseases for which vaccines have been developed, provides the explanation for the repeated failures in our attempts to stick to the current approaches (18–20). Perhaps, the current paradigm is not appropriate to develop vaccines for a virus that profoundly affects the immune system of the host and that uses many different mechanisms to escape what otherwise could be protective immune responses.

A related question is if we have in place the appropriate mechanisms to identify and support the highly innovative science that could allow for a paradigm shift? The current peer review system, which has been extremely efficient in protecting the quality of normal science, may not be the best system to stimulate out-of-the-paradigm research, with innovative concepts placed at high risk of being suppressed (71, 72). This challenge has been recognized by different institutions, leading to the creation of special initiatives to stimulate innovation on HIV vaccine research. These include the Innovation Grant Program for Approaches in HIV Vaccine Research created by in 1997 by the so-called Baltimore Committee of the National Institute of Allergy and Infectious Diseases (73, 74), the Grand Challenge on Global Health Program from the Bill & Melinda Gates Foundation (75), and the Innovation Fund from the International AIDS Vaccine Initiative (IAVI) (76). Unfortunately, those programs, that have (or had) their own mechanisms to select and monitor projects, by and large have failed to spur

the necessary innovation. The problem has been that it is very difficult to predict what innovative projects will work, or even to suggest any specific areas of exploration. However, what it is possible is to formally establish innovative processes and mechanisms to support such research.

In 2007, the Wellcome Trust and the Bill & Melinda Gates Foundation convened a meeting in London to discuss the need to bring additional innovation to HIV vaccine research. The group recognized that innovative proposals are high-risk, that peer review is conservative and risk adverse, and that peer-review can't deal with proposals that challenge accepted thinking. In considering innovative research, the group recommended that funders consider using broad-minded people who look at impact and at the big picture, ask thoughtful questions, and give applicants a chance to reply (77).

It is fair to say that that innovative HIV vaccine concepts that are not part of the mainstream thinking are regularly published. Very often those articles are initially rejected in more prestigious journals, and the authors usually struggle to secure the funds needed to advance the research and to eventually confirm and expand, or to refute the original observations. For instances, in 2012 Jean-Marie Andrieu and collaborators reported that oral immunization of chinese Rhesus macaques with a combination of *Lactobacilus plantarum* and inactivated SIV provided strong protection against subsequent infection with the virus (78, 79). More surprisingly, the observed protection did not correlate with any known adaptive immune response, but instead it correlated with CD8+ regulatory T cells that seemed to mediate a tolerogenic mechanism that falls outside of the current paradigm. Not surprisingly, the authors experienced difficulties in getting the paper accepted by different journals, and the results were received with a great deal of skepticism. Fortunately, the Bill & Melinda Gates Foundation was able to support an independent confirmation of those observations. At least in this case, a potentially game-changing idea was not dismissed *a priori,* and the results of the potentially confirmatory study will be available at the end of 2015.

Although it is beyond my individual predictive abilities to identify what could be the most promising out-of-the-paradigm concepts, a few additional examples could be listed for further exploration. One is the use of HIV-1 gp-41 subunit virosomes, which have been shown to be protective in non-human primate models, with protection correlating with mucosal antibodies rather than with circulating neutralizing antibodies (80–83). Another vaccine concept, also based on a gp41 peptide, was reported to protected CD+ T cells from lysis by natural killer cells, without having any protective effect against the infection *per se* (84, 85). Finally, although whole inactivated vaccines were extensively tested in the past in animal models, with negative results, perhaps it is not unreasonable to revisit this concept using current experimental approaches, including low-dose repeated challenges (86).

The barriers to accept new concepts or paradigms cannot be underestimated. This was well understood by the German theoretical physicist Max Planck when he said that "a new scientific truth does not triumph by convincing its opponent and making them see the light, but rather because its opponents eventually die, and a new generation grows up that is familiar with it" (from Max Planck' Scientific Autobiography, cited by Kuhn) (1).

DO WE NEED A NEW SCIENTIFIC PARADIGM TO FINALLY DEVELOP AN HIV VACCINE?

The answer to that question is: perhaps. The important point is that, while we continue pursuing the current approaches, we should also actively explore new avenues, leaving no stone unturned in our search for an HIV vaccine (87).

After all, 30 years of intense HIV vaccine research has not resulted in a practical effective vaccine, although such vaccine is sorely needed to bring the HIV epidemic under control (52). In order to accelerate the development of an HIV vaccine, we recently proposed a number of actions, including the suggestion to establish a program of truly innovative research with protected funding to explore out-of-the-paradigm approaches, perhaps allocating to this program not less than 10% of the total HIV vaccine investment (22). Innovative research, especially out of the paradigm frame, needs to be supported by an innovation ecosystem which should include, not only the innovative scientists, but also an enlightened leadership in the field, the appropriate mechanism for the selection of projects and, perhaps more importantly, a supportive scientific community (88).

HIV vaccine research needs to continue with the sense of urgency that the severity of the AIDS pandemic is imposing on us. We constantly need to keep in mind that the objective of our research is not only the acquisition of new knowledge, but the developing of a practical solution for one of the worse public health problems of our time.

ACKNOWLEDGMENTS

The author thanks Marc van Regenmortel for providing constructing criticisms and helpful comments.

REFERENCES

1. Kuhn TS. *The Structure of Scientific Revolutions.* Chicago: University of Chicago Press (1962).
2. Richmond PA. The germ theory of disease. *Henry E Sigerist Suppl Bull Hist Med* (1980) **4**:84–98.
3. Smith KA. Louis pasteur, the father of immunology? *Front Immunol* (2012) **3**:68. doi:10.3389/fimmu.2012.00068
4. Gallo RC. The discovery of the first human retroviruses: HTLV-1 and HTLV-2. *Retrovirology* (2005) **2**:17. doi:10.1186/1742-4690-2-S1-S17
5. Poiesz BJ, Ruscetti FW, Gazdar AF, Bunn PA, Minna JD, Gallo RC. Detection and isolation of type C retrovirus particles from fresh and cultured lymphocytes of a patient with cutaneous T-cell lymphomas. *Proc Nat Acad Sci U S A* (1980) **77**:7415–9. doi:10.1073/pnas.77.12.7415
6. DeVita VT. A perspective on the war on cancer. *Cancer J* (2002) **5**:352–6. doi:10.1097/00130404-200209000-00002
7. Judson HC. *The Eight Day of Creation – the Makers of the Revolution in Biology.* New York: Simon and Schuster (1979).
8. Rott R. The post-Loeffler-Frosch era: contribution of German virologists. In: Calisher CH, Horzinek MC, editors. *100 Years of Virology: The Birth and Growth of a Discipline.* Vienna: Springer (1999). p. 43–61.
9. Schäfer W. Structure of some animal viruses and significance of their components. *Bacteriol Rev* (1963) **27**:1–17.
10. Ihle JN, Collins JJ, Lee JC, Fischinger PJ, Moenning V, Schäfer W, et al. Characterization of the immune response to the major glycoprotein (gp 71) of Friend leukemia virus. I. Response in BALB/c mice. *Virology* (1976) **75**:74–87. doi:10.1016/0042-6822(76)90008-8
11. Bolognesi DP, Fischinger PJ. Prospects for treatment of human retrovirus-associated diseases. *Cancer Res* (1985) **45**:470s–5s.
12. Fischinger PJ, Gallo RC, Bolognesi DP. Toward a vaccine against AIDS: rationale and current progress. *Mt Sinai J Med* (1986) **53**:639–47.
13. Robey WG, Arthur LO, Matthews TJ, Langlois A, Copeland TD, Lerche NW, et al. Prospects for prevention of human immunodeficiency virus infection: purified

120-kDa envelope glycoprotein induces neutralizing antibody. *Proc Natl Acad Sci U S A* (1986) **83**:7023–7. doi:10.1073/pnas.83.18.7023
14. Valenzuela P, Medina A, Rutter WJ, Ammerer G, Hall BD. Synthesis and assembly of hepatitis B virus surface antigen particles in yeast. *Nature* (1982) **298**:347–50. doi:10.1038/298347a0
15. McAleer WC, Bynak EB, Maigetter RZ, Wampler DE, Miler WJ, Hileman MR. Human hepatitis from recombinant yeast. *Nature* (1984) **307**:178–80. doi:10.1038/307178a0
16. Fischinger PJ, Robey WG, Koprowsky H, Gallo RC, Bolognesi D. Current status and strategies for vaccines against diseases induced by human T-cell lymphotropic retroviruses (HTLV-I, -II, -III). *Cancer Res* (1985) **45**(9 Suppl):4694s–9s.
17. Esparza J. A brief history of the global effort to develop a preventive HIV vaccine. *Vaccine* (2013) **31**:3502–18. doi:10.1016/j.vaccine.2013.05.018
18. Esparza J, Russell N, McCutchan F. The long road to an HIV vaccine. In: Kleinman DL, Delbourne J, Cloud-Hansen KA, Handelsman J, editors. *Controversies in Science and Technology*. (Vol. 3), New York, NY: Mary Ann Liebert Inc Publishers (2010). p. 44–76.
19. Lewis GK, DeVico AL, Gallo RC. Antibody persistence and T-cell balance: two key factors confronting HIV vaccine development. *Proc Natl Acad Sci U S A* (2014) **111**:15614–21. doi:10.1073/pnas.1413550111
20. Carnathan DG, Wetzel KS, Yu J, Lee ST, Johnson BA, Paiardini M, et al. Activated CD4+CCR5+ T cells in the rectum predict increased SIV acquisition in SIVGag/Tat-vaccinated rhesus macaques. *Proc Natl Acad Sci U S A* (2015) **112**:518–23. doi:10.1073/pnas.1407466112
21. Fouts TR, Bagley K, Prado IJ, Bobb KL, Schwartz JA, Xu R, et al. Balance of cellular and humoral immunity determines the level of protection by HIV vaccines in rhesus macaque models of HIV infection. *Proc Natl Acad Sci U S A* (2015) **112**(9):E992–9. doi:10.1073/pnas.1423669112
22. Esparza J. What has 30 years of HIV vaccine research taught us? *Vaccines* (2013) **1**:513–26. doi:10.3390/vaccines1040513
23. Flynn NM, Forthal DN, Harro CD, Judson FN, Mayer KH, Para MF, et al. Placebo-controlled phase 3 trial of a recombinant glycoprotein 120 vaccine to prevent HIV-1 infection. *J Infect Dis* (2005) **191**:654–65. doi:10.1086/428404
24. Pitisuttithum P, Gilbert P, Gurwith M, Heyward W, Martin M, van Griensven F. Randomized, double-blind, placebo-controlled efficacy trial of a bivalent recombinant glycoprotein 120 HIV-1 vaccine among injection drug users in Bangkok, Thailand. *J Infect Dis* (2006) **194**:1661–71. doi:10.1086/508748
25. Buchbinder SP, Mehrotra DV, Duerr A, Fitzgerald DW, Mogg R, Li D, et al. Efficacy assessment of a cell-mediated immunity HIV-1 vaccine (the Step Study): a double-blind, randomised, placebo-controlled, test-of-concept trial. *Lancet* (2008) **372**:1881–93. doi:10.1016/S0140-6736(08)61591-3
26. Rerks-Ngarm S, Pitisuttithum P, Nitayaphan S, Kaewkungwal J, Chiu J, Paris R, et al. Vaccination with ALVAC and AIDSVAX to prevent HIV-1 infection in Thailand. *N Engl J Med* (2009) **361**:2209–20. doi:10.1056/NEJMoa0908492
27. Bolognesi DP, Matthews TJ. HIV vaccines viral envelope fails to deliver? *Nature* (1999) **391**:638–9. doi:10.1038/35504
28. Shaw GM, Hunter E. HIV transmission. *Cold Spring Harb Perspect Med* (2012) **2**(11):1–23. doi:10.1101/cshperspect.a006965
29. Nkolola JP, Bricault CA, Cheung A, Shields J, Perry J, Kovacs JM, et al. Characterization and immunogenicity of a novel mosaic M HIV-1 gp140 trimer. *J Virol* (2014) **88**:9538–52. doi:10.1128/JVI.01739-14
30. Yasmeen A, Ringe R, Derking R, Cupo A, Julien JP, Burton DR, et al. Differential binding of neutralizing and non-neutralizing antibodies to native-like soluble HIV-1 Env trimers, uncleaved Env proteins, and monomeric subunits. *Retrovirology* (2014) **11**:41. doi:10.1186/1742-4690-11-41
31. Javaherian K, Langlois AJ, McDanal C, Ross KL, Eckler LI, Jellis CL, et al. Principal neutralizing domain of the human immunodeficiency virus type 1 envelope protein. *Proc Natl Acad Sci U S A* (1989) **86**:6768–72. doi:10.1073/pnas.86.17.6768
32. Li D, Forrest BD, Li Z, Xue P, Hanson CV, Duan S, et al. International clinical trials of HIV vaccines: II. Phase I trial of an HIV-1 synthetic peptide vaccine evaluating an accelerated immunization schedule in Yunnan, China. *Asian Pac J Allergy Immunol* (1997) **15**:105–13.
33. Steimer KS, Scandella CJ, Skiles PV, Haigwood NL. Neutralization of divergent HIV-1 isolates by conformation-dependent human antibodies to gp120. *Science* (1991) **254**:105–8. doi:10.1126/science.1718036
34. Kwong PD, Mascola JR, Nabel GJ. Rational design of vaccines to elicit broadly neutralizing antibodies to HV-1. *Cold Spring Harb Perspect Med* (2011) **1**(1):a007278. doi:10.1101/cshperspect.a007278
35. Burton DR, Ahmed R, Barouch DH, Butera ST, Crotty S, Godzik A, et al. A blueprint for HIV vaccine discovery. *Cell Host Microbe* (2012) **12**:396–407. doi:10.1016/j.chom.2012.09.008
36. McLellan JS, Correia BE, Chen M, Yang Y, Graham BS, Schief WR, et al. Design and characterization of epitope-scaffold immunogens that present the motavizumab epitope from respiratory syncytial virus. *J Mol Biol* (2011) **409**:853–66. doi:10.1016/j.jmb.2011.04.044
37. Van Regenmortel MH. Basic research in HIV vaccinology is hampered by reductionist thinking. *Front Immunol* (2012) **3**:194. doi:10.3389/fimmu.2012.00194
38. Van Regenmortel MH. An outdated notion of antibody specificity is one of the major detrimental assumptions of the structure-based reverse vaccinology paradigm, which prevented it from helping to develop an effective HIV-1 vaccine. *Front Immunol* (2014) **5**:593. doi:10.3389/fimmu.2014.00593
39. Mascola JR, Haynes BF. HIV-1 neutralizing antibodies: understanding nature's pathways. *Immunol Rev* (2013) **254**:225–44. doi:10.1111/imr.12075
40. Haynes BF, Kelsoe G, Harrison SC, Kepler TB. B-cell-lineage immunogen design in vaccine development with HIV-1 as a case study. *Nat Biotechnol* (2012) **30**:423–33. doi:10.1038/nbt.2197
41. Ahlers JD. All eyes on the next generation of HIV vaccines: strategies for inducing a broadly neutralizing antibody response. *Discov Med* (2014) **17**:187–99.
42. Prabakaran P, Chen W, Dimitrov DS. The antibody germline/maturation hypothesis, elicitation of broadly neutralizing antibodies against HIV-1 and cord blood IgM repertoires. *Front Immunol* (2014) **5**:398. doi:10.3389/fimmu.2014.00398
43. Stephenson KE, Barouch DH. A global approach to HIV-1 vaccine development. *Immunol Rev* (2013) **254**:295–304. doi:10.1111/imr.12073
44. Barouch DH, Stephenson KE, Borducchi EN, Smith K, Stanley K, McNally AG, et al. Protective efficacy of a global HIV-1 mosaic vaccine against heterologous SHIV challenges in rhesus monkeys. *Cell* (2013) **155**:531–9. doi:10.1016/j.cell.2013.09.061
45. Barouch DH, Picker LJ. Novel vaccine vectors for HIV-1. *Nat Rev Microbiol* (2014) **12**:765–71. doi:10.1038/nrmicro3360
46. Teigler JE, Phogat S, Franchini G, Hirsch VM, Michael NL, Barouch DH. The canarypox virus vector ALVAC induces distinct cytokine responses compared to the vaccinia virus-based vectors MVA and NYVAC in rhesus monkeys. *J Virol* (2014) **88**:1809–14. doi:10.1128/JVI.02386-13
47. Perdiguero B, Gómez CE, Cepeda V, Sánchez-Sampedro L, García-Arriaza J, Mejías-Pérez E, et al. Virological and immunological characterization of novel NYVAC-based HIV/AIDS vaccine candidates expressing clade C trimeric soluble gp140(ZM96) and Gag(ZM96)-Pol-Nef(CN54) as VLPs. *J Virol* (2014) **89**(2):970–88. doi:10.1128/JVI.02469-14
48. Hansen SG, Ford JC, Lewis MS, Ventura AB, Hughes CM, Coyne-Johnson L, et al. Profound early control of highly pathogenic SIV by an effector memory T-cell vaccine. *Nature* (2011) **473**:523–7. doi:10.1038/nature10003
49. Hansen SG, Sacha JB, Hughes CM, Ford JC, Burwitz BJ, Scholz I, et al. Cytomegalovirus vectors violate CD8+ T cell epitope recognition paradigms. *Science* (2013) **340**:1237874. doi:10.1126/science.1237874
50. Picker LJ, Hansen SG, Lifson JD. New paradigms for HIV/AIDS vaccine development. *Annu Rev Med* (2012) **63**:95–111. doi:10.1146/annurev-med-042010-085643
51. Picker LJ. Are effector memory T cells the key to an effective HIV/AIDS vaccine? *EMBO Rep* (2014) **15**:820–1. doi:10.15252/embr.201439052
52. Fauci AS, Folkers GK, Marston HD. Ending the global HIV/AIDS pandemic: the critical role of an HIV vaccine. *Clin Infect Dis* (2014) **59**(Suppl 2):S80–4. doi:10.1093/cid/ciu420
53. Burton DR, Desrosiers RC, Doms RW, Feinberg MB, Gallo RC, Hahn B, et al. Public health. A sound rationale needed for phase III HIV-1 vaccine trials. *Science* (2004) **303**:316. doi:10.1126/science.1094620
54. Haynes BF, Gilbert PB, McElrath MJ, Zolla-Pazner S, Tomaras GD, Alam SM, et al. Immune-correlates analysis of an HIV-1 vaccine efficacy trial. *N Engl J Med* (2012) **366**:1275–8. doi:10.1056/NEJMoa1113425
55. Yates NL, Liao HX, Fong Y, deCamp A, Vandergrift NA, Williams WT, et al. Vaccine-induced Env V1-V2 IgG3 correlates with lower HIV-1 infection risk and declines soon after vaccination. *Sci Transl Med* (2014) **6**:228ra39. doi:10.1126/scitranslmed.3007730

56. Veillette M, Désormeaux A, Medjahed H, Gharsallah NE, Coutu M, Baalwa J, et al. Interaction with cellular CD4 exposes HIV-1 envelope epitopes targeted by antibody-dependent cell-mediated cytotoxicity. *J Virol* (2014) **88**:2633–44. doi:10.1128/JVI.03230-13
57. Bonsignori M, Pollara J, Moody MA, Alpert MD, Chen X, Hwang KK, et al. Antibody-dependent cellular cytotoxicity-mediating antibodies from an HIV-1 vaccine efficacy trial target multiple epitopes and preferentially use the VH1 gene family. *J Virol* (2012) **86**:11521–32. doi:10.1128/JVI.01023-12
58. DeVico A, Fouts T, Lewis GK, Gallo RC, Godfrey K, Charurat M, et al. Antibodies to CD4-induced sites in HIV gp120 correlate with the control of SHIV challenge in macaques vaccinated with subunit immunogens. *Proc Natl Acad Sci U S A* (2007) **104**:17477–82. doi:10.1073/pnas.0707399104
59. Guan Y, Pazgier M, Sajadi MM, Kamin-Lewis R, Al-Darmarki S, Flinko R, et al. Diverse specificity and effector function among human antibodies to HIV-1 envelope glycoprotein epitopes exposed by CD4 binding. *Proc Natl Acad Sci U S A* (2013) **110**:E69–78. doi:10.1073/pnas.1217609110
60. Gómez-Román VR, Patterson LJ, Venzon D, Liewehr D, Aldrich K, Florese R, et al. Vaccine-elicited antibodies mediate antibody-dependent cellular cytotoxicity correlated with significantly reduced acute viremia in rhesus macaques challenged with SIVmac251. *J Immunol* (2005) **174**:2185–9. doi:10.4049/jimmunol.174.4.2185
61. Moldt B, Schultz N, Dunlop DC, Alpert MD, Harvey JD, Evans DT, et al. A panel of IgG1 b12 variants with selectively diminished or enhanced affinity for Fcγ receptors to define the role of effector functions in protection against HIV. *J Virol* (2011) **85**:10572–81. doi:10.1128/JVI.05541-11
62. Excler JL, Ake J, Robb ML, Kim JH, Plotkin SA. Nonneutralizing functional antibodies: a new "old" paradigm for HIV vaccines. *Clin Vaccine Immunol* (2014) **21**:1023–36. doi:10.1128/CVI.00230-14
63. Hammer SM, Sobieszczyk ME, Janes H, Karuna ST, Mulligan MJ, Grove D, et al. Efficacy trial of a DNA/rAd5 HIV-1 preventive vaccine. *N Engl J Med* (2013) **369**:2083–92. doi:10.1056/NEJMoa1310566
64. P5 Partnership. Available from: http://www.vaccineenterprise.org/content/P5Partnership (2014).
65. Robb ML, Rerks-Ngarm S, Nitayaphan S, Pitisuttithum P, Kaewkungwal J, Kunasol P, et al. Risk behaviour and time as covariates for efficacy of the HIV vaccine regimen ALVAC-HIV (vCP1521) and AIDSVAX B/E: a post-hoc analysis of the Thai phase 3 efficacy trial RV 144. *Lancet Infect Dis* (2012) **12**:531–7. doi:10.1016/S1473-3099(12)70088-9
66. Esparza J. Understanding the efficacy variables of an HIV vaccine trial. *Lancet Infect Dis* (2012) **12**:499–500. doi:10.1016/S1473-3099(12)70117-2
67. Bregu M, Draper SJ, Hill AV, Greenwood BM. Accelerating vaccine development and deployment: report of a Royal Society satellite meeting. *Philos Trans R Soc Lond B Biol Sci* (2011) **366**:2841–9. doi:10.1098/rstb.2011.0100
68. Nathanson N, Mathieson BJ. Biological considerations in the development of a human immunodeficiency virus vaccine. *J Infect Dis* (2000) **182**:579–89. doi:10.1086/315707
69. Medawar PB. *Advice to a Young Scientist*. New York: Harper Colophon Books (1979).
70. Miedema F. A brief history of HIV vaccine research: stepping back to the drawing board? *AIDS* (2008) **22**:1699–703. doi:10.1097/QAD.0b013e3283021a61
71. Kaplan D. Point: statistical analysis in NIH peer review – identifying innovation. *FASEB J* (2007) **21**:305–8. doi:10.1096/fj.07-0204ufm
72. Kaplan D, Lacetera N, Kaplan C. Sample size and precision in NIH peer review. *PLoS One* (2008) **3**:e2761. doi:10.1371/journal.pone.0002761
73. Parrott C. First grants for innovative AIDS vaccine research awarded. *NIAID AIDS Agenda* (1997):5–7.
74. Culliton BJ. "Innovation" in AIDS vaccines. *Nat Med* (1997) **3**:1181. doi:10.1038/nm1097-1057a
75. Varmus H, Klausner R, Zerhouni E, Acharya T, Daar AS, Singer PA. Public health. Grand challenges in global health. *Science* (2003) **302**:398–9. doi:10.1126/science.1091769
76. McEnery R. The AIDS vaccine field considers ways to encourage innovation and recruit new minds to the effort. *IAVI Rep* (2009) **13**:9–13.
77. Goodwin P. In: Report from the Wellcome-Gates Innovation Meeting. London (2007).
78. Lu W, Chen S, Lai C, Guo W, Fu L, Andrieu JM. Induction of CD8+ regulatory T cells protects macaques against SIV challenge. *Cell Rep* (2012) **2**:1736–46. doi:10.1016/j.celrep.2012.11.016
79. Andrieu JM, Chen S, Lai C, Guo W, Lu W. Mucosal SIV vaccines comprising inactivated virus particles and bacterial adjuvants induce CD8(+) T-regulatory cells that suppress SIV-positive CD4(+) T-cell activation and prevent SIV infection in the macaque model. *Front Immunol* (2014) **5**:297. doi:10.3389/fimmu.2014.00297
80. Bomsel M, Tudor D, Drillet AS, Alfsen A, Ganor Y, Roger MG, et al. Immunization with HIV-1 gp41 subunit virosomes induces mucosal antibodies protecting nonhuman primates against vaginal SHIV challenges. *Immunity* (2011) **34**:269–80. doi:10.1016/j.immuni.2011.01.015
81. Leroux-Roels G, Maes C, Clement F, van Engelenburg F, van den Dobbelsteen M, Adler M, et al. Randomized phase I: safety, immunogenicity and mucosal antiviral activity in young healthy women vaccinated with HIV-1 Gp41 P1 peptide on virosomes. *PLoS One* (2013) **8**(2):e55438. doi:10.1371/journal.pone.0055438
82. McElrath MJ. Standing guard at the mucosa. *Immunity* (2011) **34**:146–8. doi:10.1016/j.immuni.2011.02.014
83. Zhou M, Ruprecht RM. Are anti-HIV IgAs good guys or bad guys? *Retrovirology* (2014) **11**:109. doi:10.1186/PREACCEPT-1729873135143527
84. Vieillard V, Strominger JL, Debré P. NK cytotoxicity against CD4+ T cells during HIV-1 infection: a gp41 peptide induces the expression of an NKp44 ligand. *Proc Natl Acad Sci U S A* (2005) **102**:10981–6. doi:10.1073/pnas.0504315102
85. Plotkin SA. Problems in vaccine development. *Monoclon Antib Immunodiagn Immunother* (2014) **33**:166–7. doi:10.1089/mab.2014.0011
86. Sheppard HW, Dorman BP. Time for a systematic look at inactivated HIV vaccines. *AIDS* (2015) **29**:125–7. doi:10.1097/QAD.0000000000000476
87. Esparza J, Van Regenmortel MH. More surprises in the development of an HIV vaccine. *Front Immunol* (2014) **5**:329. doi:10.3389/fimmu.2014.00329
88. Van Regenmortel MHV, Andrieu J-M, Dimitrov DS, Ensoli B, Hioe CE, Moog C, et al. Paradigm changes and the future of HIV vaccines: a summary of a workshop held in Baltimore on 20 November 2013. *J AIDS Clin Res* (2014) **5**:281. doi:10.4172/2155-6113.1000281

Conflict of Interest Statement: The author declares that the research was conducted in the absence of any commercial or financial relationship that could be construed as a potential conflict of interest.

*Received: 06 January 2015; accepted: 06 March 2015; published online: 18 March 2015. Citation: Esparza J (2015) A new scientific paradigm may be needed to finally develop an HIV vaccine. Front. Immunol. **6**:124. doi: 10.3389/fimmu.2015.00124*
This article was submitted to HIV and AIDS, a section of the journal Frontiers in Immunology.

frontiers in
IMMUNOLOGY

OPINION ARTICLE
published: 16 July 2014
doi: 10.3389/fimmu.2014.00335

Design challenges for HIV-1 vaccines based on humoral immunity

Neil S. Greenspan*

Department of Pathology, Case Western Reserve University, Cleveland, OH, USA
**Correspondence: nsg@case.edu*

Edited by:
Marc H. V. Van Regenmortel, University of Strasbourg, France

Reviewed by:
Quentin James Sattentau, University of Oxford, UK
Marc H. V. Van Regenmortel, University of Strasbourg, France

Keywords: genomic diversity, immune escape, broadly neutralizing antibody, non-neutralizing antibody, vaccine-guided B cell evolution, immunogen design, regulatory CD8$^+$ T cells, vectored immunoprophylaxis

INTRODUCTION

Human Immunodeficiency Virus-1 (HIV-1) presents profound challenges to vaccine developers. Potential hurdles of relevance to the design of an effective HIV-1 vaccine based on humoral immunity include: (1) the exceptional rate of mutation of the viral genome due to an error-prone polymerase (i.e., reverse transcriptase) (1), (2) a relatively high rate of genomic recombination (2, 3), (3) both within-host and between-host evolution (4), (4) extensive glycosylation of the chief antigenic target (gp120) recognized by potentially protective antibodies (5, 6), (5) immunodominance of regions of the envelope glycoprotein that display a high degree of primary structure diversity (7) favoring the generation of neutralizing antibodies of narrow breadth, (6) a relatively low density of antigen spikes on the virion surface (8, 9) thereby minimizing multivalent antibody binding and perhaps raising the threshold affinity required for potent neutralization by at least some antibodies, (7) the ability of HIV to infect CD4$^+$ T cells and other cells critical to functioning of the immune system, and deplete the numbers of these cells, and (8) perhaps most insidious, activation of CD4$^+$ T cells, which is necessary for the generation of potent broadly neutralizing antibodies in response to immunization may simultaneously increase the number of cells susceptible to infection by HIV (10, 11).

The scale of the antigenic diversity characterizing the HIV-1 envelope molecules, which are the primary targets of antibody-mediated immunity, is truly daunting. According to Korber et al. (1), the HIV-1 viral genomes in one infected individual encompass the same approximate extent of nucleotide sequence diversity that is exhibited by the worldwide population of influenza A viral genomes over the course of a year.

While most HIV transmission events appear to trace back to a single virus, up to a quarter of infections may involve infection by two to five viruses (12). So, even if vaccine immunization elicits a robust antibody response, the probability that the antibodies circulating in the blood of a vaccinated individual will effectively neutralize or otherwise mediate immunity against all of the viruses mediating infection will be greatest if those antibodies are broadly neutralizing. In addition, due to rapid within-host evolution, substantially different viruses may be infecting different individuals in a large population. Therefore, antibodies generated by vaccination will need to be broadly neutralizing to achieve high levels of vaccine efficacy.

UNIQUE FEATURES OF BROADLY NEUTRALIZING ANTIBODIES FOR HIV-1

Investigators interested in HIV-1 vaccines based on humoral immunity have established that most neutralizing antibodies in most patients neutralize a limited range of strains, primarily those to which the individual patient was exposed or closely related strains. In contrast, only about 25–30% of patients synthesize potent and broadly neutralizing antibodies (pbnAb) and typically only after 2–4 years following the initiation of infection. This time interval for the development of protective antibodies is exceptionally long.

The extent of somatic hypermutation focused on the rearranged immunoglobulin (Ig) genes encoding the heavy and light chain variable domains and that has been associated with broad neutralizing activity against HIV-1 is also extraordinary. Ig genes encoding typical anti-pathogen antibodies from a mature immune response may exhibit on the order of 15–20 mutations in the gene segments encoding the heavy chain variable domain. In contrast, many pbnAb for HIV-1 have 40–100 somatic mutations in the genes encoding the heavy chain variable domain. It seems plausible to infer that a truly exceptional extent of Ig gene evolution is contributory to and possibly essential for the generation of at least some pbnAb. This inference is supported by the recent findings that many pbnAb contain many heavy chain V domain-encoding mutations in framework regions and these mutations, at both contact and non-contact positions, are necessary for both high potency and great breadth of neutralization activity (13). Although, the high number of variable domain mutations in pbnAb may also reflect in part the rapid pace of within-host HIV evolution, this possibility does not obviously account for the typical and rather extended time frame observed for the development of pbnAb.

In either case, it will also be important to study the critical role of follicular helper CD4$^+$ cells (14) in driving proliferation as well as somatic hypermutation and affinity maturation in germinal center B cells. Another useful focus for future investigation is based on the

recent insights regarding the neutralizing activities of glycan-specific Ab (15).

Thus, it is fair to question whether the standard approach to clinical vaccination of administering the same immunogen several times is likely to be effective in the case of HIV-1. A number of major laboratories investigating this problem have already begun fashioning novel approaches in which the ultimate overall goal is likely to be immunization with a series of related but distinguishable HIV-1-derived envelope molecules that guide the evolutionary trajectory for the genes encoding the variable (V) domains of the corresponding neutralizing antibodies (16). Such a scheme would appear to recapitulate to some extent the *in vivo* process that occurs for B cells in infected patients. The logistics of vaccine delivery entailed by this sort of immunization scheme may be more challenging than for any previously successful clinical vaccine.

A crucial feature of this approach of guiding B cell evolution to the production of pbnAb reflects the troubling fact that the epitope recognized by a protective Ab at the end of the process may not be bound by the B cell receptors (BCR) of the germline B cells from which such antibodies will ultimately be derived by clonal evolution (16). Therefore, vaccine developers have begun working to identify antigens suitable for the activation of the B cells expressing germline BCRs that can serve as the ancestral sequences for pbnAb.

This sort of approach is undoubtedly made more plausible by recent and impressive advances in structure-guided methods for designing vaccine immunogens to contain specific epitopes (16, 17). The results of Dey et al. and Jardine et al. suggest the potential of these new techniques for eliciting antibody responses of desired specificity for the HIV-1 envelope protein.

REMAINING QUESTIONS REGARDING IMMUNITY ELICITED BY VACCINE-GUIDED B CELL EVOLUTION

Nevertheless, it remains to be determined: (1) if identification of monoclonal pbnAb can lead to designed vaccine immunogens that reliably elicit protective polyclonal serum or mucosal antibodies, (2) how many sequential immunogens will be required to reliably guide the evolutionary trajectory to pbnAb in the great majority of vaccine recipients, (3) how many total immunizations will be needed, (4) what intervals between immunizations would achieve the optimal balance between immunogenicity and a timeframe for elicitation of protective responses compatible with public health goals, and (5) how much expense such a scheme, implemented on a mass scale, will entail.

The preceding strategy is based on the assumption that the most viable approach to generating effective immunity to HIV-1 is to elicit pbnAb by an active humoral immune response. There is evidence that non-neutralizing antibodies may be able to contribute to immunity to the virus (18–20), but it appears that only a minority of investigators focusing on HIV vaccines based on humoral immunity are persuaded that such immunity can be of sufficient potency to protect vaccine recipients in the absence of pbnAb. It is of importance of determine if elicitation of non-neutralizing antibodies to HIV-1 antigens is a feasible path to successful protection in a high percentage of vaccine recipients.

REGULATING CD4$^+$ T CELLS AS A PATH TO IMMUNITY TO HIV-1

A counter-intuitive approach to vaccine development, although not based in elicitation of humoral immunity, merits brief discussion. Since HIV-1 infects CD4$^+$ T cells (which are crucial for the sorts of humoral immune responses we address above), and susceptibility to HIV-1 is increased after activation through the T cell receptor, Lu et al. (10) reasoned that an immunogen able to diminish responses of CD4$^+$ T cells might actually reduce susceptibility of a recipient of that immunogen to HIV infection. These authors demonstrated that administration of an oral vaccine consisting of an inactivated simian immunodeficiency virus (SIVmac239) plus the tolerance-inducing commensal bacterium *Lactobacillus plantarum* to macaques protected them from subsequent intrarectal challenge. CD8$^+$ T cell-depleting antibodies confirmed the necessary role of CD8$^+$ regulatory T cells to this unusual form of vaccine-mediated immunity to infection.

In the macaque model of SIV infection described by Lu et al., the beneficial effect of reduced responsiveness by CD4$^+$ T cells was mediated by CD8$^+$ T cells recognizing antigens presented by non-classical MHC class I molecules. It will obviously be of interest to determine if the same cell type could operate similarly in humans, assuming the phenomenon is reproducible across species. Another important question that merits investigation is whether this approach to vaccination is effective in eliciting protection against infection for virus entry by other routes. Even if in humans, a vaccine targeting CD8$^+$ T cells recognizing antigens presented by non-classical MHC class I molecules were able to provide protection from HIV infection, it would not necessarily rule out the possibility of manipulating standard CD4$^+$ regulatory T cells to add to any benefit associated with CD8$^+$ regulatory T cells.

VECTORED IMMUNOPROPHYLAXIS AS AN APPROACH TO HUMORAL IMMUNITY

Another approach to generating humoral immunity to HIV-1 is based not on induction of active B cell immunity but on vectored immunoprophylaxis (21, 22), In this strategy, a viral vector (e.g., adeno-associated virus, AAV) is used to infect or otherwise insert genes encoding intact potent broadly neutralizing antibodies into host cells *ex vivo* with subsequent implantation or *in vivo*. This scheme provides arguably passive immunity in that there is no administration of an immunogen related to HIV and no elicitation of an immune response involving host B lymphocytes, as usually defined. However, the production of antibodies is active in the host and no already synthesized antibodies are directly infused. Early studies in animal models have demonstrated proof of principle for using vectored immunoprophylaxis to confer robust protection to recipient animals challenged with significant doses of virulent HIV by clinically relevant routes (21, 22).

Of course, applying vectored immunoprophylaxis on a mass scale as an alternative to standard vaccination also requires addressing so far unanswered questions. Who should receive the treatment and at what age? Is a single administration of the vector sufficient for long-lasting antibody production and protection? Are periodic administrations of the vector needed to maintain persistent protective immunity? Could HIV evolve so as to escape one or even multiple pbnAb generated

via vectored immunoprophylaxis? If antibody produced by the vector produced undesirable side-effects, how could synthesis be abrogated in a timely manner? Will the genetic vectors persist in treated patients for periods and in ways that cause unwelcome side-effects? Can proponents of vectored immunoprophylaxis produce data that will assure the FDA that these safety concerns have been adequately addressed?

CONCLUSION

Due to a constellation of attributes including enormous genomic and antigenic sequence diversity, rapid evolution, and immunity-subverting structural features of key antigens associated with HIV-1, it is one of the most challenging pathogens vaccine developers have confronted to date. Technological advances in cloning Ig genes from individual human B lymphocytes, generating human monoclonal antibodies, and designing immunogens to express one or a limited number of epitopes have been extraordinary and rapid. These advances make plausible a vaccination scheme centered on the notion of using a series of related but non-identical immunogens to guide the process of B cell evolution through repeated rounds of somatic hypermutation leading to affinity maturation and acquisition of broadly neutralizing activity. However, numerous scientific and logistical challenges remain to be addressed before such a scheme could be implemented on a public health scale. Therefore, alternative strategies to generating protective immunity to HIV-1, such as vectored immunoprophylaxis or induction of regulatory responses intended to reduce activation of $CD4^+$ T cells, remain worthy of thorough exploration.

ACKNOWLEDGMENTS

Neil Greenspan is Professor of Pathology in the Case Western Reserve University School of Medicine in Cleveland, OH, USA and was supported by NIH grant U19 AI-091031 and the CWRU/UHCMC Center for AIDS Research and NIH grant number P30 AI036219. The author thanks Marc van Regenmortel for the invitation to contribute an article on the challenges associated with HIV vaccine development and for sharing his perspectives on the topic, and the reviewers for their thoughtful and valuable comments.

REFERENCES

1. Korber B, Gaschen B, Yusim K, Thakallapally R, Kesmir C, Detours V. Evolutionary and immunological implications of contemporary HIV-1 variation. *Br Med Bull* (2001) **58**:19–42. doi:10.1093/bmb/58.1.19
2. Burke DS. Recombination in HIV: an important viral evolutionary strategy. *Emerg Infect Dis* (1997) **3**:253–9. doi:10.3201/eid0303.970301
3. Lemey P, Rambaut A, Pybus OG. HIV evolutionary dynamics within and among hosts. *AIDS Rev* (2006) **8**:125–40.
4. Alizon S, Fraser C. Within-host and between-host evolutionary rates across the HIV-1 genome. *Retrovirology* (2013) **10**:49. doi:10.1186/1742-4690-10-49
5. Wyatt R, Kwong PD, Desjardins E, Sweet RW, Robinson J, Hendrickson WA, et al. The antigenic structure of the HIV gp120 envelope glycoprotein. *Nature* (1998) **393**:705–11. doi:10.1038/31514
6. Wei X, Decker JM, Wang S, Hui H, Kappes JC, Wu X, et al. Antibody neutralization and escape by HIV-1. *Nature* (2003) **422**(6929):307–12. doi:10.1038/nature01470
7. Phogat S, Wyatt R. Rational modifications of HIV-1 envelope glycoproteins for immunogen design. *Curr Pharm Des* (2007) **13**:213–27. doi:10.2174/138161207779313632
8. Chertova E, Bess JW Jr, Crise BJ, Sowder IIRC, Schaden TM, Hilburn JM, et al. Envelope glycoprotein incorporation, not shedding of surface envelope glycoprotein (gp120/SU), is the primary determinant of SU content of purified human immunodeficiency virus type 1 and simian immunodeficiency virus. *J Virol* (2002) **76**:5315–25. doi:10.1128/JVI.76.11.5315-5325.2002
9. Zhu P, Liu J, Bess J Jr, Chertova E, Lifson JD, Grisé H, et al. Distribution and three-dimensional structure of AIDS virus envelope spikes. *Nature* (2006) **441**:847–52. doi:10.1038/nature04817
10. Lu W, Chen S, Lai C, Guo W, Fu L, Andrieu JM. Induction of $CD8^+$ regulatory T cells protects macaques against SIV challenge. *Cell Rep* (2012) **2**:1736–46. doi:10.1016/j.celrep.2012.11.016
11. Fauci AS, Marston HD. Ending AIDS – is an HIV vaccine necessary? *N Engl J Med* (2014) **370**:495–8. doi:10.1056/NEJMp1313771
12. Keele BF, Giorgi EE, Salazar-Gonzalez JF, Decker JM, Pham KT, Salazar MG, et al. Identification and characterization of transmitted and early founder virus envelopes in primary HIV-1 infection. *Proc Natl Acad Sci U S A* (2008) **105**:7552–7. doi:10.1073/pnas.0802203105
13. Klein F, Diskin R, Scheid JF, Gaebler C, Mouquet H, Georgiev IS, et al. Somatic mutations of the immunoglobulin framework are generally required for broad and potent HIV-1 neutralization. *Cell* (2013) **153**:126–38. doi:10.1016/j.cell.2013.03.018
14. Gitlin AD, Shulman Z, Nussenzweig MC. Clonal selection in the germinal centre by regulated proliferation and hypermutation. *Nature* (2014) **509**:637–40. doi:10.1038/nature13300
15. Sok D, Doores KJ, Briney B, Le KM, Saye-Francisco KL, Ramos A, et al. Promiscuous glycan site recognition by antibodies to the high-mannose patch of gp120 broadens neutralization of HIV. *Sci Transl Med* (2014) **6**:236ra63. doi:10.1126/scitranslmed.3008104
16. Dey B, Svehla K, Xu L, Wycuff D, Zhou T, Voss G, et al. Structure-based stabilization of HIV-1 gp120 enhances humoral immune responses to the induced co-receptor binding site. *PLoS Pathog* (2009) **5**:e1000445. doi:10.1371/journal.ppat.1000445
17. Jardine J, Julien JP, Menis S, Ota T, Kalyuzhniy O, McGuire A, et al. Rational HIV immunogen design to target specific germline B cell receptors. *Science* (2013) **340**:711–6. doi:10.1126/science.1234150
18. Baum LL, Cassutt KJ, Knigge K, Khattri R, Margolick J, Rinaldo C, et al. HIV-1 gp120-specific antibody-dependent cell-mediated cytotoxicity correlates with rate of disease progression. *J Immunol* (1996) **157**:2168–73.
19. Lambotte O, Ferrari G, Moog C, Yates NL, Liao HX, Parks RJ, et al. Heterogeneous neutralizing antibody and antibody-dependent cell cytotoxicity responses in HIV-1 elite controllers. *AIDS* (2009) **23**:897–906. doi:10.1097/qad.0b013e328329f97d
20. Haynes BF, Gilbert PB, McElrath MJ, Zolla-Pazner S, Tomaras GD, Alam SM, et al. Immune-correlates analysis of an HIV-1 vaccine efficacy trial. *N Engl J Med* (2012) **366**:1275–86. doi:10.1056/NEJMoa1113425
21. Balazs AB, Chen J, Hong CM, Rao DS, Yang L, Baltimore D. Antibody-based protection against HIV infection by vectored immunoprophylaxis. *Nature* (2011) **481**:81–4. doi:10.1038/nature10660
22. Balazs AB, Ouyang Y, Hong CM, Chen J, Nguyen SM, Rao DS, et al. Vectored immunoprophylaxis protects humanized mice from mucosal HIV transmission. *Nat Med* (2014) **20**:296–300. doi:10.1038/nm.3471

Conflict of Interest Statement: The author declares that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Received: 31 May 2014; accepted: 03 July 2014; published online: 16 July 2014.
Citation: Greenspan NS (2014) Design challenges for HIV-1 vaccines based on humoral immunity. Front. Immunol. 5:335. doi: 10.3389/fimmu.2014.00335
This article was submitted to HIV and AIDS, a section of the journal Frontiers in Immunology.

frontiers in
IMMUNOLOGY

REVIEW ARTICLE
published: 18 November 2014
doi: 10.3389/fimmu.2014.00593

An outdated notion of antibody specificity is one of the major detrimental assumptions of the structure-based reverse vaccinology paradigm, which prevented it from helping to develop an effective HIV-1 vaccine

Marc H. V. Van Regenmortel*†

CNRS, Biotechnologie des Interactions Moleculaires, IREBS, School of Biotechnology, ESBS, University of Strasbourg, Illkirch, France

Edited by:
Kendall A. Smith, Weill Medical College of Cornell University, USA

Reviewed by:
Gianfranco Pancino, Institut national de la santé et de la recherche médicale (INSERM), France
Frank Miedema, University Medical Centre Utrecht, Netherlands

***Correspondence:**
Marc H. V. Van Regenmortel, Pole API, Boulv Sebastien Brant, BP 10413, Illkirch cedex, France
e-mail: vanregen@unistra.fr

†***Present address:***
Marc H. V. Van Regenmortel, CNRS, UMR7242 – Institut de Recherche de l'Ecole de Biotechnologie de Strasbourg (IREBS), Université de Strasbourg, Illkirch, France

The importance of paradigms for guiding scientific research is explained with reference to the seminal work of Karl Popper and Thomas Kuhn. A prevalent paradigm, followed for more than a decade in HIV-1 vaccine research, which gave rise to the strategy known as structure-based reverse vaccinology is described in detail. Several reasons why this paradigm did not allow the development of an effective HIV-1 vaccine are analyzed. A major reason is the belief shared by many vaccinologists that antibodies possess a narrow specificity for a single epitope and are not polyspecific for a diverse group of potential epitopes. When this belief is abandoned, it becomes obvious that the one particular epitope structure observed during the crystallographic analysis of a neutralizing antibody–antigen complex does not necessarily reveal, which immunogenic structure should be used to elicit the same type of neutralizing antibody. In the physical sciences, scientific explanations are usually presented as logical deductions derived from a relevant law of nature together with certain initial conditions. In immunology, causal explanations in terms of a single cause acting according to a law of nature are not possible because numerous factors always play a role in bringing about an effect. The implications of this state of affairs for the rational design of HIV vaccines are outlined. An alternative approach to obtain useful scientific understanding consists in intervening empirically in the immune system and it is suggested that manipulating the system experimentally is needed to learn to control it and achieve protective immunity by vaccination.

Keywords: structure-based reverse vaccinology, HIV-1 vaccines, antibody polyspecificity, paradigm, immunological explanations

> "It makes no sense to do the same thing over and over again and expect a different result."
>
> Albert Einstein

INTRODUCTION

The development of science is promoted when scientists adhere to so-called paradigms that correspond to theoretical presuppositions and assumptions that guide the lines of investigation they pursue. When trying to solve a particular scientific puzzle, for instance how to develop an effective HIV-1 vaccine, the paradigm will also commit scientists to using particular experimental approaches and tools believed to be essential for finding a solution to the problem.

The importance of paradigms in guiding scientific research was proposed by Thomas Kuhn (1) in his influential book "*The Structure of Scientific Revolutions*" published in 1962. Unfortunately, he never clearly defined the concept of paradigm and he used the term in various ways, for instance, to refer to a collection of procedures and ideas that instruct scientists what to believe and how to work (2). Kuhn later conceded that the term paradigm had become hopelessly overused because it came to signify virtually any dominant idea that binds a scientific community together. He subsequently recommended that *paradigm* be replaced by *exemplar* to mean exemplary instances of successful puzzle-solutions for scientific problems. An exemplar captures the way in which a theory or model is believed to solve a problem while at the same time defining, which new problems could be addressed in a similar way. However, the term paradigm was never abandoned.

Kuhn argued that when scientists in the course of their work obtain results that contradict the theory or hypothesis that gave rise to a paradigm, they do not conclude that the paradigm has been refuted and must be abandoned. Scientists, therefore, do not follow the injunction of Karl Popper that their aim should be to try to disprove or falsify their theories rather than prove them. Popper maintained that observations are never able to prove a theory but can only sometimes logically refute a mistaken theory (3). He argued that when scientists obtain reproducible results that are at odds with their working hypothesis, they are logically obliged to accept that the hypothesis has been falsified and they should therefore abandon it (4). Kuhn disagreed and claimed that this is not the way scientists behave because their main commitment is not to test or seek to confirm the implicit theories and hypotheses that

underlie the paradigms they adhere to. Scientists in fact tend to ignore anomalous results and will devise new *ad hoc* hypotheses in an effort to explain away apparent contradictions between theory and experimental observations. Kuhn further claimed that science can make progress only if scientific communities remain committed to their shared theoretical beliefs and experimental techniques and do not abandon a paradigm or hypothesis as soon as incompatible results are obtained (5). Only if troublesome anomalies keep accumulating over many years may scientists eventually start questioning their presuppositions and lose their confidence in a given paradigm. This could then usher a scientific revolution that occurs when a paradigm is superseded by a new one and gives rise to a paradigm shift. Periods of so-called normal science are then replaced by a short period of revolutionary science (1).

In HIV vaccine research, there is evidence that several prevalent paradigms have not helped the development of an effective vaccine (6–8). One such paradigm, which gave rise to the strategy known as structure-based reverse vaccinology (RV) (9) was pursued vigorously for more than a decade although it did not lead to the development of an effective HIV-1 vaccine. The theoretical underpinnings of this paradigm have been discussed previously because they illustrate the need for investigators to question the implicit underlying assumptions that make them pursue unfruitful lines of investigation (10, 11). Only when the presuppositions or hypotheses that gave rise to unsuccessful paradigms are shown to be invalid will investigators become aware that a paradigm shift is required in a particular scientific field (8).

STRUCTURE-BASED RV PARADIGM IN HIV-1 VACCINE RESEARCH

The approach known as RV was introduced in the field of bacterial vaccines by Rino Rappuoli (12, 13) and refers to the strategy of predicting potential vaccine immunogens using bioinformatics analyses of entire bacterial genomes in order to identify all the surface-exposed proteins that a bacterial pathogen is able to express. The strategy is called RV because investigators operate in a reverse manner, i.e., starting from the genome rather than from the organism, to discover, which bacterial proteins should be studied as potential vaccine immunogens. This allows hundreds of bacterial proteins to be identified as candidate immunogens even when bacteria cannot be cultivated and bacterial extracts cannot therefore be fractionated to establish empirically which proteins are able to induce a protective immune response.

In virology, RV has a different meaning and refers to a strategy, which attempts to generate a vaccine from a knowledge of protective antibodies (Abs) rather than from the usual reverse task of generating such Abs by immunization with a vaccine (9, 14). It was suggested that effective vaccine immunogens might be discovered by exploring the interaction of anti-HIV-1 neutralizing (n)Abs with HIV-1 envelope (Env) spikes, using X-ray crystallography of Env–Ab complexes. This expectation was based on the assumption that once the 3D structure of a broadly neutralizing monoclonal antibody (bnMab) bound to an Env epitope had been elucidated, it would be possible to use the bnMab as a template to reconstruct its complementary epitope outside the context of the natural Env antigen, using structure-based design. It was further hypothesized that this reconstructed epitope designed to fit the bnMab would possess the immunogenic capacity of inducing a polyclonal Ab response with the same neutralizing capacity as the bnAb used as template. The assumption was that if an HIV-1 epitope is able to bind an Ab, it will also possess the immunogenic capacity to elicit the same type of Ab in an immunized host. That this is not necessarily the case, however, is demonstrated by the common observation that when a peptide fragment of a protein is able to bind Abs raised against the protein, the peptide will frequently be unable to elicit Abs that react with the native protein (15).

Since the RV approach used to develop bacterial vaccines has been highly successful (13) whereas, the RV approach used in the HIV-1 vaccine field has failed so far (10, 16, 17) it has been suggested that the two approaches should be clearly differentiated and could be called genome-based RV and structure-based RV, respectively (18).

UNWARRANTED ASSUMPTIONS OF THE STRUCTURE-BASED RV PARADIGM

The structure-based RV approach suffers from several unwarranted expectations that jeopardized the ability of developing an effective HIV-1 vaccine. It did not clearly distinguishing between antigenicity and immunogenicity (12, 19) and analyzed epitopes in antigens and paratopes in Abs solely in terms of which amino acids in the two partners made contact with each other (20). As a result, little attention was given to the fact that the binding activity of an Ab often depends on structural features distant from the paratope itself (21, 22) and that residues in the antigen that are not in contact with the paratope may nevertheless be able to affect the binding activity and the immunogenic activity of epitopes (23, 24). Such findings confirm an insight reached years ago that paratopes and epitopes defined in terms of contact residues often differ from the binding sites defined in functional assays (25). The structures visualized in Ab-antigen complexes also tend to differ from the structures of the binding sites in the free molecules, before they have been altered by the mutual adaptation and induced fit that occur when the two partners interact (26, 27). This means that the particular antigenic structure revealed in an Ab–antigen complex does not necessarily correspond to the immunogenic structure that was recognized by B-cell receptors (BCRs) during the immunization process and which therefore is often presumed to be needed in the vaccine immunogen.

In an earlier review (11), more than 50 original science publications were mentioned, which attempted to reconstruct active HIV-1 epitopes using conformational constraints, protein scaffolds, and other structure-based engineering approaches. Although some of the engineered epitopes possessed increased antigenicity and reacted better with bnMabs, none of them were found to be effective vaccine immunogens, illustrating the shortcomings of the structure-based RV approach (28–32).

OVERLOOKING THE NEED FOR ANTIBODY AFFINITY MATURATION TO OBTAIN EFFECTIVE ANTI HIV-1 NEUTRALIZING Abs

Another unjustified hypothesis of the structure-based RV approach was that the HIV-1 epitopes recognized by the matured bnMabs that are present in HIV-1 infected individuals after a

lengthy process of Ab affinity maturation will be able to trigger a protective immune response in naïve individuals. However, studies involving the deep sequencing of all the HIV-1 Abs present in the serum of infected individuals demonstrated that the initial immunogen triggering the Ab affinity maturation process that leads to neutralizing Abs usually recognizes a germline version of BCRs that differs considerably from the BCRs corresponding to mature bnAbs (33, 34). This means that the epitopes engineered by structure-based RV to mimic HIV-1 epitopes recognized by mature bnMabs are unlikely to be effective vaccine immunogens because they are mostly unable to recognize the germline BCRs present in naïve individuals (35–38).

The extent of affinity maturation observed in HIV-1 bnAbs is much more extensive than the 5–10% mutation frequency in the Ab hypervariable regions observed with Abs directed to other viruses (37–39) and this characteristic feature of HIV-1 bnAbs together with the enormous antigenic variability of the virus explains why the structure-based RV approach in the case of HIV-1 presented unsurmountable challenges compared to other viral vaccines (36, 40, 41). HIV-1 elicits bnAbs only in a minority of infected individuals after several years of infection and attempts are currently made to unravel the mutational pathway that leads from germline BCR ancestors to mature Abs (42). The goal is to identify various immunogens capable of stimulating successive B-cell responses through multiple rounds of antibody maturation processes (43, 44). This is clearly no mean task since large numbers of maturation pathways are likely to exist (45–47).

Current attempts to modify Env epitopes so that they are able to bind germline BCRs or maturation intermediates depart from the original structure-based RV paradigm because they no longer attempt to directly transform epitopes of known structure recognized by mature bnMabs into immunogens capable of eliciting similar bnAbs (46–48). This new approach represents a new paradigm based on the germline/maturation hypothesis (34), which assumes that it may be possible to discover effective HIV-1 vaccine immunogens that bind putative germline antibody predecessors of known HIV-1 bnAbs although they do not bind the highly somatically mutated bnAbs themselves (49).

In recent years, increasing numbers of bnMabs have been isolated from HIV-1 infected individuals (50–52) and it has been suggested that these Mabs provide valuable molecular information that could inform HIV-1 vaccine design (53, 54). Such bnMabs may be of value for passive immunotherapy since they could provide sterilizing immunity to humans and non-human primates when they are administered prior to viral challenge (55, 56). However, their usefulness does not at present extend to the vaccine field since we have no idea how Abs that possess the protective capacity of such bnMabs can be induced by vaccination (11). There is also considerable evidence that a protective immune response requires the combined neutralizing activities of several Abs that target different non-overlapping HIV-1 Env epitopes (57) as well as Abs that act in synergy (58–60). What may be required, therefore, is to find immunogens able to elicit classical types of protective, polyclonal immune responses rather than elusive immunogens that would elicit single Ab specificities endowed with the exceptional neutralizing capacity of individual bnMabs such as the well-studied b12, VRC01, PG9, or PG16 bnMabs (61–63).

RELIANCE ON REDUCTIONIST THINKING: ANOTHER PITFALL OF THE STRUCTURE-BASED RV PARADIGM

Another pitfall of the structure-based RV paradigm is its reliance on reductionist thinking (11). Reductionism has been prevalent in molecular biology for half a century and is still popular today because it has been very successful for dissecting biological systems into their constituent parts (64, 65). The reductionist mindset made immunologists accept that the biological activities of Abs could be explained by their 3D structures and that the immunogenic potential of a viral epitope could be deduced from its antigenic properties. Biological immunogenicity was thereby reduced to chemical antigenicity, which is a variation of the claim that biology can be reduced to chemistry (66). Such a claim fails to recognize that the protection achieved by vaccination is a biological phenomenon that has meaning only in the context of an entire organism since organs, tissues, or molecules cannot be vaccinated. Protection always results from a complex network of dynamic interactions between pathogen, host, and immune system and it cannot be satisfactorily understood when innumerable, individual molecular interactions are analyzed separately.

The use of Mabs also introduces a reductionist bias in the analysis of protein antigenicity and immunogenicity because it leads investigators to focus on artificial boundaries between overlapping epitopes and to ignore the fact that the apparent immunological specificity of a Mab very much depends on the selection process that was used to obtain it. Since a Mab is always polyspecific, the fact that it binds, for instance, to one particular peptide of the membrane proximate external region (MPER) of HIV-1 gp41 simply reflects the fact that the Mab was selected for its ability to bind to that peptide. Such a Mab may, however, recognize better a more complex epitope of gp41 that could actually have been the immunogen that gave rise to the Mab. If it is assumed that the Mab was elicited by the linear MPER peptide epitope because it binds to it, this may induce investigators to only investigate peptides as possible vaccine immunogens, a choice, which is likely to be self-defeating (15, 67).

STRUCTURE-BASED RV PARADIGM SUFFERS FROM ANOTHER MAJOR MISGUIDED ASSUMPTION, NAMELY AN OUTDATED NOTION OF Ab SPECIFICITY

According to this view, which is usually not explicitly acknowledged, a Mab that would bind for instance to an epitope on the HIV-1 Env surface is believed to be able to recognize only a single defined target area (the so-called complementary epitope of the Mab) corresponding to the surface residues found to be in contact with paratope residues in the Ab–antigen complex. Although the belief that Mabs are monospecific for a single epitope is no longer held and was shown to be invalid already 30 years ago (68–74) many vaccinologists have been slow to accept that there is no single intrinsic or specific epitope for any Ab but only a diverse group of potential epitopes able to bind to it with various degrees of fit (10, 75, 76). It seems that the failure to recognize that Abs are always polyspecific and possess considerable plasticity, promiscuity, degeneracy, and cross-reactive potential has made the structure-based RV approach appear more plausible to many investigators (10).

It is now well-established that most Abs derived from Ab germline genes and expressed before any antigenic stimulation are highly polyreactive and react with a wide variety of autoantigens such as DNA, cytoskeleton proteins, nuclear antigens, and carbohydrates as well as numerous bacterial and viral antigens (70, 77–81). The various mechanisms that allow a polyspecific Ab to recognize a multiplicity of epitopes and antigens have been elucidated (82). Once investigators realize that the epitope structure observed in a bnMab–HIV-1 Env complex is only one of the many epitopes that could be accommodated by what is always a polyspecific Ab, they have no difficulty to accept that any one of these epitopes could correspond to the immunogen that gave rise to the Mab. They will then no longer assume that the one epitope structure observed in the crystallographic analysis of an antigen complex must necessarily reveal, which immunogenic structure should be used to elicit such a bnAb (10).

Furthermore, the fact that Abs are not only polyspecific but are also heterospecific, i.e., able to react more strongly with other antigens than with the immunogen used for eliciting the Ab, also helps to clarify why antigenic and immunogenic properties are not always simultaneously present in the same region of a protein (76). The immunogenic capacity of an epitope to induce heterospecific Abs that do not react with the protein used for immunization demonstrates that immunogenicity need not be accompanied by an antigenic reactivity that enables the epitope to bind to the induced Abs. Similarly, the antigenic reactivity of a viral protein revealed by its ability to bind a given polyspecific Ab is also not necessarily accompanied by an immunogenic capacity to induce that same Ab in a particular immune system (76).

Although the advent of Mabs has completely transformed our ability to dissect immune responses to proteins, their utilization has also introduced a bias in the antigenic analysis of viruses because investigators tend to focus on artificial boundaries between overlapping epitopes and overlook the fact that the surface of a protein is an antigenic and functional continuum (83). When an nMab has been isolated, it tends to become associated with a unique, discrete epitope, which leads to the expectation that a vaccine containing this epitope will induce similar nAbs. However, most protective immune responses are polyclonal and involve the collective and synergistic neutralizing activities of Abs directed to different epitopes (61). When the therapeutic efficacy of mixtures of HIV-1 neutralizing Mabs was tested in HIV-1 infected humanized mice, it was found that mixtures of five Mabs were more effective than single Mabs or mixtures of three Mabs (84). Mixtures of five Abs suppressed viral loads below the level of detection and also failed to select escape viral mutants. However, chronically HIV-1 infected humans from whom bnAbs were isolated do not appear to benefit from such Abs for controlling virus replication (17, 85, 86), nor do HIV-1 infected long-term progressors compared to non-progressors (87). The role of Abs in controlling chronic HIV-1 infection therefore remains an issue that should be further investigated.

Many unsuccessful attempts have been made to elicit by immunization bnAbs similar to potent bnMabs isolated from HIV-1 infected individuals (17, 88). The past research emphasis on unraveling the neutralization mechanisms of individual bnMabs did not help because it did not provide any information on which immunogens were capable of eliciting the different types of bnAbs. The poor success rate so far in discovering even one such effective HIV-1 vaccine immunogen by structure-based RV is certainly a cause of concern. As discussed elsewhere (8), there is a clear need to study new vaccine immunogens and new methods to induce strong mucosal antibody responses using for instance specific adjuvants. Other markers than viral load and CD4 T cell counts for assessing vaccine efficacy should also be investigated (8).

It is nowadays commonly claimed that rational design offers the best prospects for developing an HIV-1 vaccine and that this approach is superior to the empirical screening and trial-and-error strategies used in the past. When it is claimed that "rational design represents the only approach that can elevate vaccine research from an empirical exercise to a scientific discipline" (89), the essential contribution of empirical trials to vaccine development is actually denigrated as if trial-and-error experimentation were not an entirely rational enterprise (10, 11, 19). When authors discuss the rational design of an HIV-1 vaccine (90, 91) they only refer to studies that improve the degree of complementarity in a single epitope–Mab pair and they do not clarify how an improved antigen could actually be "designed" to also become an immunogen capable of inducing protective Abs. Optimizing the binding activity of a viral antigen by structure-based design using a single Mab as a template is certainly feasible but this is not equivalent to immunogen design, which requires the intentional optimization of numerous factors extrinsic to the epitope–paratope recognition such as the various cellular and regulatory mechanisms of the host that exist only in the context of the vaccinated host and control the generation of neutralizing Abs (11, 92). Antigen design is simply masquerading as immunogen design when it is assumed that an improved viral antigen will also be an effective vaccine immunogen capable of inducing a protective immune response. The so-called rational design of vaccine immunogens by mimicking the rational approach used in drug design (10, 93, 94) is not feasible without extensive empirical clinical trials of vaccine candidates and to suggest otherwise contradicts the well-established empirical nature of vaccine science. The concern that too much funding may be diverted to empirical clinical trials at the expense of basic structure-based HIV vaccine research (95) seems unwarranted since the lion share of current funding is devoted to structural studies whereas, the required small scale clinical trials based on innovative paradigm concepts remain poorly funded (8).

CAUSES, EXPLANATIONS, AND UNDERSTANDING IN VACCINOLOGY

The selection of a paradigm to guide the empirical search for an HIV-1 vaccine always depends on framing hypotheses about causal mechanisms, which could provide a plausible explanation for a possible successful vaccine. Most scientific explanations take the form of a causal mechanistic explanation, which means that causes, explanations and understanding are usually intimately linked (96, 97). It is relevant therefore to analyze how these three terms are used in vaccinology.

In the physical sciences, explanations are usually presented as logical deductions derived from one or a few relevant laws of nature, together with certain initial conditions. However, this is not feasible in the biological sciences because of the absence of

universal biological laws (11, 98). Since the probability of a biological event is always affected by a very large number of causal factors, causal links become diluted and it is usually not possible to provide an explanation in vaccinology in terms of a single cause.

The reductionist dissection of the immune system into its components severs the dynamic connections that link the parts of a biological complex system in a functionally integrated manner. This allows any level in the resulting biological complexity to be the starting point of a causal analysis, provided a certain state of affairs is considered to be in need of an explanation. As a result, explanations are usually framed in terms of a complex type of probabilistic causality that attempts to take into account the numerous factors that together contribute to an effect in a given biological context. This means that the classical notion of causality is of limited value for providing explanations in immunology (11, 96). The contribution of one causal factor in a complex multicausal biological system can actually only be investigated by altering that factor experimentally and assessing whether the observed effect is then no longer the same in a given context (94). This has led to the suggestion that many biological phenomena may be too complex to be comprehended or explained by human intelligence (99).

Attempts to achieve understanding in immunology often start with information about observed effects and by an awareness that certain phenomena share underlying similarities, which then leads one to propose a theory or hypothetical model to explain them. The structure-based RV paradigm is one such model, which assumes that bnMabs that are isolated from HIV-1 infected individuals and recognize particular Env epitopes are likely to have been elicited by these epitopes. When the model was tested by assessing the immunogenicity of the epitopes recognized by different bnMabs, it was found that the results did not fit the model and that no bnAbs were elicited (29, 30, 100). This should remind us that a proposed explanatory model does not predict the data to which it is fitted since the model is actually chosen to fit the data and it makes no sense to fit what one wants to explain (101). Understanding consists of knowledge about relations of dependence and should make it possible to derive inferences about the consequences of our interventions and give us an ability to predict and control phenomena. Unfortunately, it seems that scientists tend to overestimate the detail and depth of their understanding, which often consists of nothing more than an informed guess about the future prospects of their scientific work. This leads to a frame of mind that has been called an "illusion of depth of understanding in science" (102). In the absence of experimental data that back their explanatory model or paradigm, scientists do not choose which theory to accept but choose which theory they are actually going to work with. Scientists are then driven by the promise of future understanding rather than by past convincing explanatory evidence and such an expectation is easily influenced by wishful thinking about their pet theories (102).

Since understanding a phenomenon is ultimately displayed by: (1) making right predictions, (2) successfully intervening in a system, and (3) answering explanation-seeking questions about it, the inability to do any of these things is a clear indication that alternative explanatory hypotheses and paradigms are required. As far as HIV-1 vaccines are concerned, our ignorance of why all Env immunogens investigated so far have been unable to induce adequate levels of potent protective Abs is a clear indication that we do not understand the complex mechanisms that are involved in achieving protective immunity against HIV-1.

Although the complexity of the immune system may prevent us from identifying all its internal regulatory mechanisms, it is by comparing the various ways of manipulating the system using empirical experimentation that we may eventually control it and achieve protective immunity by vaccination (11).

Empirical, scientific knowledge is based on experimental and observational facts and on the rule that "nothing trumps experience." However, it has been argued that empirical evidence only allows scientists to draw plausible but tentative conclusions when the obtained results make it possible to successfully control and manipulate the experimental system under investigation (103, 104). If this does not happen, empiricism dictates that we should investigate additional constituents of the complex biological systems we study until we improve our ability to predict the results of our experimental interventions. It is indeed the ability of investigators to successfully intervene in a material system that gives them the knowledge needed to manipulate and control it.

Since the aim of biologists is both to explain and control biological phenomena, explaining goes hand-in-hand with intervening and it has been suggested that "explanations in biology are always obtained through direct intervention on models of the phenomenon to be explained" (105). It is important to recognize that such a view is at odds with the widespread expectation that we will succeed in developing an effective HIV-1 vaccine only when we have significantly increased our general knowledge of basic immunology and of HIV-1 antigenic structure (11). This means that we need to interfere with the material world in order to obtain empirical knowledge about it and that our scientific understanding increases when we are able to successfully manipulate the system we investigate (106).

CONCLUSION

There is evidence that the popular structure-based RV paradigm used in HIV-1 vaccine research has not been helpful for developing an effective HIV-1 vaccine. Some of the reasons for this lack of success are summarized, one of them being the failure to recognize that all Abs as well as bnMabs isolated from HIV-1 infected individuals are always polyspecific and able to bind to a variety of related and unrelated epitopes. Since the epitope structure identified by X-ray crystallography of a bnMab–HIV-1 Env complex is only one of several epitopes that could be accommodated by the Mab, there is no reason to assume that this epitope of known structure must correspond to the immunogen that elicited the antibody.

Recently, one of the major groups committed to the structure-based RV paradigm reported a study of bnMabs that recognize a high-mannose epitope patch centered on the N 332 residue on HIV Env. They demonstrated that these Abs actually did not have a single defined target point at N 332 but were in fact polyspecific and able to bind various glycan patches as well as a glycan site located at N334 (107). For the first time, these authors admitted that antibody polyspecificity was a relevant concept in HIV-1 vaccine research and they claimed that their results represent an extension of the concept of antibody promiscuity and degeneracy

that has been widely accepted in immunology for many years (10, 68, 73–75). They also concluded that polyclonal Abs are more effective for neutralizing viruses than individual Mabs and that the polyspecificity of vaccine-induced Abs should receive increased attention. The new insights arrived at by these authors might in time alter the expectations of the proponents of the structure-based RV paradigm and diminish their reliance on structural data derived from the study of individual bnMabs.

The structure-based RV paradigm has been followed by several large networks of investigators who operate under the strong leadership of principal investigators (108). This situation leads to considerable built-in inertia and does not encourage funding agencies to back large numbers of high-risk projects based on alternative innovative paradigms that could diversify the vaccine strategies that are investigated (8, 108). These issues are currently receiving increased attention and it is hoped that this will in due course lead to increased funding for new original science based on novel paradigms and that it will stimulate a rigorous evaluation of existing HIV vaccine programs (108).

REFERENCES

1. Kuhn TS. *The Structure of Scientific Revolutions*. Chicago: University of Chicago Press (1962).
2. Horgan J. *The End of Science*. New York, NY: Broadway Books (1996). p. 41–7.
3. Popper K. *The Logic of Scientific Discovery*. London: Hutchinson (1959).
4. Horgan J. *The End of Science*. New York, NY: Broadway Books (2006). p. 32–41.
5. Bird A. Thomas Kuhn. Fall 2013 ed. In: Zalta EN, editor. *The Stanford Encyclopedia of Philosophy*. (2013). Available from: http://plato.stanford.edu/entries/thomas-kuhn/
6. Esparza J. The global HIV vaccine enterprise. *Int Microbiol* (2005) **8**:93–101.
7. Esparza J. What has 30 years of HIV research taught us? *Vaccines* (2013) **1**:513–26. doi:10.3390/vaccines1040513
8. Van Regenmortel MHV, Andrieu J-M, Dimitrov DS, Ensoli B, Hioe CE, Moog C, et al. Paradigm changes and the future of HIV vaccine research: a summary of a workshop held in Baltimore on 20 November 2013. *J AIDS Clin Res* (2014) **5**:281. doi:10.4172/2155-6113.1000281
9. Burton DR. Antibodies, viruses and vaccines. *Nat Rev Immunol* (2002) **2**:706–13. doi:10.1038/nri891
10. Van Regenmortel MHV. Requirements for empirical immunogenicity trials, rather than structure-based design, for developing an effective HIV vaccine. *Arch Virol* (2012) **157**:1–20. doi:10.1007/s00705-011-1145-2
11. Van Regenmortel MHV. Basic research in HIV vaccinology is hampered by reductionist thinking. *Front Immunol* (2012) **3**:194. doi:10.3389/fimmu.2012.00194
12. Rappuoli R. Reverse vaccinology, a genome-based approach to vaccine development. *Vaccine* (2001) **19**:2688–91. doi:10.1016/S0264-410X(00)00554-5
13. Rappuoli R, Bagnoli F. *Vaccine Design*. Norfolk: Carster Academic Press (2011).
14. Walker LM, Burton DR. Rational antibody-based HIV-1 vaccine design: current approaches and future directions. *Curr Opin Immunol* (2010) **22**:358–66. doi:10.1016/j.coi.2010.02.012
15. Van Regenmortel MHV. Synthetic peptide vaccines and the search for neutralization of B cell epitopes. *Open Vaccin J* (2009) **2**:33–44. doi:10.2174/1875035401002010033
16. Burton DR, Ahmed R, Barouch DH, Butera ST, Crotty S, Godzik A, et al. A blueprint for HIV vaccine discovery. *Cell Host Microbe* (2012) **12**:396–407. doi:10.1016/j.chom.2012.09.008
17. Corti D, Lanzavecchia A. Broadly neutralizing antiviral antibodies. *Ann Rev Immunol* (2013) **31**:705–42. doi:10.1146/annurev-immunol-032712-095916
18. Van Regenmortel MHV. Two meanings of reverse vaccinology and the empirical nature of vaccine science. *Vaccine* (2011) **29**:7875. doi:10.1016/j.vaccine.2011.08.063
19. Kong L, Sattentau QJ. Antigenicity and immunogenicity in HIV-1 antibody-based vaccine design. *J AIDS Clin Res* (2012) **8**:3. doi:10.4172/2155-6113.S8-003
20. Sundberg EJ, Mariuzza RA. Molecular recognition in antibody-antigen complexes. *Adv Protein Chem* (2002) **61**:119–60. doi:10.1016/S0065-3233(02)61004-6
21. Schildbach JF, Near RI, Bruccoleri RE, Haber E, Jeffrey PD, Novotny J, et al. Modulation of antibody affinity by a non-contact residue. *Protein Sci* (1993) **2**:206–14. doi:10.1002/pro.5560020209
22. Chatellier J, Van Regenmortel MHV, Vernet T, Altschuh D. Functional mapping of conserved residues located at the VL and VH domain interface of a Fab. *J Mol Biol* (1996) **264**:1–6. doi:10.1006/jmbi.1996.0618
23. Greenspan NS, DiCera E. Defining epitopes: it's not as easy as it seems. *Nat Biotechnol* (1999) **17**:936–7. doi:10.1038/13590
24. Moudgil KD, Sercarz EE, Grewal IS. Modulation of the immunogenicity of antigenic determinants by their flanking residues. *Immunol Today* (1998) **19**:217–20. doi:10.1016/S0167-5699(97)01233-4
25. Cunningham BC, Wells JA. Comparison of a structural and a functional epitope. *J Mol Biol* (1993) **234**:554–63. doi:10.1006/jmbi.1993.1611
26. Wilson IA, Stanfield RL. Antibody-antigen interactions: new structures and new conformational changes. *Curr Opin Struct Biol* (1994) **4**:857–67. doi:10.1016/0959-440X(94)90267-4
27. Bosshard HR. Molecular recognition by induced fit: how fit is the concept? *News Physiol Sci* (2001) **16**:171–3.
28. Joyce JG, Hurni WM, Bogusky MJ, Garsky VM, Liang X, Citron MP, et al. Enhancement of alpha-helicity in the HIV-1 inhibitory peptide DP178 leads to an increased affinity for human monoclonal antibody 2F5 but does not elicit neutralizing responses in vitro. Implications for vaccine design. *J Biol Chem* (2002) **277**:45811–20. doi:10.1074/jbc.M205862200
29. Ho J, Uger RA, Zwick MB, Luscher MA, Barber BH, MacDonald KS. Conformational constraints imposed on a pan-neutralizing HIV-1 antibody epitope result in increased antigenicity but not neutralizing response. *Vaccine* (2005) **23**:1559–73. doi:10.1016/j.vaccine.2004.09.037
30. Burton DR. Scaffolding to build a rational vaccine design strategy. *Proc Natl Acad Sci U S A* (2010) **107**:17859–60. doi:10.1073/pnas.1012923107
31. Pejchal R, Wilson IA. Structure-based vaccine design in HIV: blind men and the elephant? *Curr Pharm Des* (2010) **16**:3744–53. doi:10.2174/138161210794079173
32. Van Regenmortel MHV. Limitations to the structure-based design of HIV-1vaccine immunogens. *J Mol Recognit* (2011) **24**:741–53. doi:10.1002/jmr.1116
33. Xiao X, Chen W, Feng Y, Zhu Z, Prabakaran P, Wang Y, et al. Germline-like predecessors of broadly neutralizing antibodies lack measurable binding to HIV-1 envelope glycoproteins: implications for evasion of immune responses and design of vaccine immunogens. *Biochem Biophys Res Commun* (2009) **390**:404–9. doi:10.1016/j.bbrc.2009.09.029
34. Dimitrov DS. Therapeutic antibodies, vaccines and antibodyomes. *MAbs* (2010) **2**:347–56. doi:10.4161/mabs.2.3.11779
35. Zhu Z, Qin HR, Chen W, Zhao Q, Shen X, Schutte R, et al. Cross-reactive HIV-1-neutralizing human monoclonal antibodies identified from a patient with 2F5-like antibodies. *J Virol* (2011) **85**:11401–8. doi:10.1128/JVI.05312-11
36. Chen W, Streaker ED, Russ DE, Feng Y, Prabakaran P, Dimitrov D. Characterization of germline antibody libraries from human umbilical cord blood and selection of monoclonal antibodies to viral envelope glycoproteins: implications for mechanisms of immune evasion and design of vaccine immunogens. *Biochem Biophys Res Commun* (2012) **417**:1164–9. doi:10.1016/j.bbrc.2011.12.089
37. Klein F, Diskin R, Scheid JF, Gaebler C, Mouquet H, Georgiev IS, et al. Somatic mutations of the immunoglobulin framework are generally required for broad and potent HIV-1 neutralization. *Cell* (2013) **153**:126–38. doi:10.1016/j.cell.2013.03.018
38. Jardine J, Julien JP, Menis S, Ota T, Kalyuzhniy O, McGuire A, et al. Rational HIV immunogen design to target specific germline B cell receptors. *Science* (2013) **340**:711–6. doi:10.1126/science.1234150
39. Zhu Z, Bossart KN, Bishop KA, Crameri G, Dimitrov AS, McEachern JA, et al. Exceptionally potent cross-reactive neutralization of Nipah and Hendra viruses by a human monoclonal antibody. *J Infect Dis* (2008) **197**:846–53. doi:10.1086/528801
40. Burton DR, Stanfield RL, Wilson IA. Antibody vs. HIV in a clash of evolutionary titans. *Proc Natl Acad Sci U S A* (2005) **102**:14943–8. doi:10.1073/pnas.0505126102
41. McLellan JS, Chen M, Joyce MG, Sastry M, Stewart-Jones GB, Yang Y, et al. Structure-based design of a fusion glycoprotein vaccine for respiratory syncytial virus. *Science* (2013) **342**:592–8. doi:10.1126/science.1243283

42. Liao HX, Lynch R, Zhou T, Gao F, Alam SM, Boyd SD, et al. Co-evolution of a broadly neutralizing HIV-1 antibody and founder virus. *Nature* (2013) **496**:469–76. doi:10.1038/nature12053
43. Gray ES, Madiga MC, Hermanus T, Moore PL, Wibmer CK, Tumba NL, et al. The neutralization breadth of HIV-1 develops incrementally over four years and is associated with CD4+ T cell decline and high viral load during acute infection. *J Virol* (2011) **85**:4828–40. doi:10.1128/JVI.00198-11
44. Euler Z, van den Kerkhof TL, van Gils MJ, Burger JA, Edo-Matas D, Phung P, et al. Longitudinal analysis of early HIV-1-specific neutralizing activity in an elite neutralizer and in five patients who developed cross-reactive neutralizing activity. *J Virol* (2012) **86**:2045–55. doi:10.1128/JVI.06091-11
45. Wu X, Zhou T, Zhu J, Zhang B, Georgiev I, Wang C, et al. Focused evolution of HIV-1 neutralizing antibodies revealed by structures and deep sequencing. *Science* (2011) **333**:1593–602. doi:10.1126/science.1207532
46. Mouquet H, Nussenzweig MC. HIV: roadmaps to a vaccine. *Nature* (2013) **496**:441–2. doi:10.1038/nature12091
47. Haynes BF, Kelsoe G, Harrison SC, Kepler TB. B-cell-lineage immunogen design in vaccine development with HIV-1 as a case study. *Nat Biotechnol* (2012) **30**:423–33. doi:10.1038/nbt.2197
48. Fauci AS, Marston HD. Ending AIDS – is an HIV vaccine necessary? *N Engl J Med* (2014) **370**:495–8. doi:10.1056/NEJMp1313771
49. Prabakaran P, Chen W, Dimitrov DS. The antibody germline/maturation hypothesis, elicitation of broadly neutralizing antibodies against HIV-1 and cord blood IgM repertoires. *Front Immunol* (2014) **5**:398. doi:10.3389/fimmu.2014.00398
50. Scheid JF, Mouquet H, Feldhahn N, Seaman MS, Velinzon K, Pietzsch J, et al. Broad diversity of neutralizing antibodies isolated from memory B cells in HIV-infected individuals. *Nature* (2009) **458**:636–40. doi:10.1038/nature07930
51. Stamatatos L, Morris L, Burton DR, Mascola JR. Neutralizing antibodies generated during natural HIV-1 infection: good news for an HIV-1 vaccine? *Nat Med* (2009) **15**:866–70. doi:10.1038/nm.1949
52. Sattentau QJ, McMichael AJ. New templates for HIV-1 antibody-based vaccine design. *F1000 Biol Rep* (2010) **2**:60. doi:10.3410/B2-60
53. Mascola JR, Montefiori DC. The role of antibodies in HIV vaccines. *Annu Rev Immunol* (2010) **28**:413–44. doi:10.1146/annurev-immunol-030409-101256
54. Burton DR, Weiss RA. AIDS/HIV. A boost for HIV vaccine design. *Science* (2010) **329**:770–3. doi:10.1126/science.1194693
55. Mascola JR, Lewis MG, Stiegler G, Harris D, VanCott TC, Hayes D, et al. Protection of macaques against pathogenic simian/human immunodeficiency virus 89.6PD by passive transfer of neutralizing antibodies. *J Virol* (1999) **73**:4009–18.
56. Hofmann-Lehmann R, Vlasak J, Rasmussen RA, Smith BA, Baba TW, Liska V, et al. Postnatal passive immunization of neonatal macaques with a triple combination of human monoclonal antibodies against oral simian-human immunodeficiency virus challenge. *J Virol* (2001) **75**:7470–80. doi:10.1128/JVI.75.16.7470-7480.2001
57. Doria-Rose NA, Klein RM, Daniels MG, O'Dell S, Nason M, Lapedes A, et al. Breadth of human immunodeficiency virus-specific neutralizing activity in sera: clustering analysis and association with clinical variables. *J Virol* (2010) **84**:1631–6. doi:10.1128/JVI.01482-09
58. Laal S, Burda S, Gorny MK, Karwowska S, Buchbinder A, Zolla-Pazner S. Synergistic neutralization of human immunodeficiency virus type 1 by combinations of human monoclonal antibodies. *J Virol* (1994) **68**:4001–8.
59. Mascola JR. HIV/AIDS: allied responses. *Nature* (2007) **449**:29–30. doi:10.1038/449029a
60. Zwick MB, Wang M, Poignard P, Stiegler G, Katinger H, Burton DR, et al. Neutralization synergy of human immunodeficiency virus type 1 primary isolates by cocktails of broadly neutralizing antibodies. *J Virol* (2001) **75**:12198–208. doi:10.1128/JVI.75.24.12198-12208.2001
61. Burton DR, Pyati J, Koduri R, Sharp SJ, Thornton GB, Parren PW, et al. Efficient neutralization of primary isolates of HIV-1 by a recombinant human monoclonal antibody. *Science* (1994) **266**:1024–7. doi:10.1126/science.7973652
62. Walker LM, Phogat SK, Chan-Hui PY, Wagner D, Phung P, Goss JL, et al. Broad and potent neutralizing antibodies from an African donor reveal a new HIV-1 vaccine target. *Science* (2009) **326**:285–9. doi:10.1126/science.1178746
63. Wu X, Yang ZY, Li Y, Hogerkorp CM, Schief WR, Seaman MS, et al. Rational design of envelope identifies broadly neutralizing human monoclonal antibodies to HIV-1. *Science* (2010) **329**:856–61. doi:10.1126/science.1187659
64. Van Regenmortel MHV. Reductionism and the search for structure-function relationships in antibody molecules. *J Mol Recognit* (2002) **15**:240–7. doi:10.1002/jmr.584
65. Van Regenmortel MHV. Reductionism and complexity in molecular biology. Scientists now have the tools to unravel biological complexity and overcome the limitations of reductionism. *EMBO J* (2004) **5**:1016–20. doi:10.1038/sj.embor.7400284
66. Van Regenmortel MHV, Hull D. *Promises and Limits of Reductionism in the Biomedical Sciences*. Chichester: Wiley (2002).
67. Montero M, van Houten NE, Wang X, Scott JK. The membrane-proximal external region of the human immunodeficiency virus type 1 envelope: dominant site of antibody neutralization and target for vaccine design. *Microbiol Mol Biol Rev* (2008) **72**:54–84. doi:10.1128/MMBR.00020-07
68. Sperling R, Francus T, Siskind GW. Degeneracy of antibody specificity. *J Immunol* (1983) **131**:882–5.
69. Berek C, Milstein C. Mutation drift and repertoire shift in the maturation of the immune response. *Immunol Rev* (1987) **96**:23–41. doi:10.1111/j.1600-065X.1987.tb00507.x
70. Chen ZJ, Wheeler CJ, Shi W, Wu AJ, Yarboro CH, Gallagher M, et al. Polyreactive antigen-binding B cells are the predominant cell type in the newborn B cell repertoire. *Eur J Immunol* (1998) **28**:989–94. doi:10.1002/(SICI)1521-4141(199803)28:03<989::AID-IMMU989>3.0.CO;2-1
71. Parnes O. From interception to incorporation: degeneracy and promiscuous recognition as precursors of a paradigm shift in immunology. *Mol Immunol* (2004) **40**:985–91. doi:10.1016/j.molimm.2003.11.021
72. Cohen IR, Hershberg U, Solomon S. Antigen-receptor degeneracy and immunological paradigms. *Mol Immunol* (2004) **40**:993–6. doi:10.1016/j.molimm.2003.11.020
73. Notkins AL. Polyreactivity of antibody molecules. *Trends Immunol* (2004) **25**:174–9. doi:10.1016/j.it.2004.02.004
74. Eisen HN, Chakraborty AK. Evolving concepts of specificity in immune reactions. *Proc Natl Acad Sci U S A* (2010) **107**:22373–80. doi:10.1073/pnas.1012051108
75. Wucherpfennig KW, Allen PM, Celada F, Cohen IR, De Boer R, Garcia KC, et al. Polyspecificity of T cell and B cell receptor recognition. *Semin Immunol* (2007) **19**:216–24. doi:10.1016/j.smim.2007.02.012
76. Van Regenmortel MHV. Specificity, polyspecificity and heterospecificity of antibody-antigen recognition. *J Mol Recognit* (2014) **27**:627–39. doi:10.1002/jmr.2394
77. Manivel V, Bayiroglu F, Siddiqui Z, Salunke DM, Rao KV. The primary antibody repertoire represents a linked network of degenerate antigen specificities. *J Immunol* (2002) **169**:888–97. doi:10.4049/jimmunol.169.2.888
78. Yin J, Beuscher AE IV, Andryski SE, Stevens RC, Schultz PG. Structural plasticity and the evolution of antibody affinity and specificity. *J Mol Biol* (2003) **330**:651–6. doi:10.1016/S0022-2836(03)00631-4
79. Haynes BF, Moody MA, Verkoczy L, Kelsoe G, Alam SM. Antibody polyspecificity and neutralization of HIV-1: a hypothesis. *Hum Antibodies* (2005) **14**:59–67.
80. Zhou ZH, Zhang Y, Hu YF, Wahl LM, Cisar JO, Notkins AL. The broad antibacterial activity of the natural antibody repertoire is due to polyreactive antibodies. *Cell Host Microbe* (2007) **15**:51–61. doi:10.1016/j.chom.2007.01.002
81. Mouquet H, Nussenzweig MC. Polyreactive antibodies in adaptive immune responses to viruses. *Cell Mol Life Sci* (2012) **69**:1435–45. doi:10.1007/s00018-011-0872-6
82. Mariuzza RA. Multiple paths to multispecificity. *Immunity* (2006) **24**:359–61. doi:10.1016/j.immuni.2006.03.009
83. Berzofsky JA. Intrinsic and extrinsic factors in protein antigenic structure. *Science* (1985) **229**:932–40. doi:10.1126/science.2410982
84. Klein F, Halper-Stromberg A, Horwitz JA, Gruell H, Scheid JF, Bournazos S, et al. HIV therapy by a combination of broadly neutralizing antibodies in humanized mice. *Nature* (2012) **492**:118–22. doi:10.1038/nature11604
85. Doria-Rose NA. HIV neutralizing antibodies: clinical correlates and implications for vaccines. *J Infect Dis* (2010) **201**:981–3. doi:10.1086/651143
86. Euler Z, van Gils MJ, Bunnik EM, Phung P, Schweighardt B, Wrin T, et al. Cross-reactive neutralizing humoral immunity does not protect from HIV type 1 disease progression. *J Infect Dis* (2010) **201**:1045–53. doi:10.1086/651144
87. van Gils MJ, Bunnik EM, Burger JA, Jacob Y, Schweighardt B, Wrin T, et al. Rapid escape from preserved cross-reactive neutralizing humoral immunity

without loss of viral fitness in HIV-1-infected progressors and long-term non progressors. *J Virol* (2010) **84**:3576–85. doi:10.1128/JVI.02622-09
88. Euler Z, Bunnik EM, Burger JA, Boeser-Nunnink BD, Grijsen ML, Prins JM, et al. Activity of broadly neutralizing antibodies, including PG9, PG16, and VRC01, against recently transmitted subtype B HIV-1 variants from early and late in the epidemic. *J Virol* (2011) **85**:7236–45. doi:10.1128/JVI.00196-11
89. Karlsson Hedestam GB, Fouchier RA, Phogat S, Burton DR, Sodroski J, Wyatt RT. The challenges of eliciting neutralizing antibodies to HIV-1 and to influenza virus. *Nat Rev Microbiol* (2008) **6**:143–55. doi:10.1038/nrmicro1819
90. Douek DC, Kwong PD, Nabel GJ. The rational design of an AIDS vaccine. *Cell* (2006) **124**:677–81. doi:10.1016/j.cell.2006.02.005
91. Nabel GJ, Kwong PD, Mascola JR. Progress in the rational design of an AIDS vaccine. *Philos Trans R Soc Lond B Biol Sci* (2011) **366**:2759–65. doi:10.1098/rstb.2011.0096
92. Van Regenmortel MHV. Molecular design versus empirical discovery in peptide-based vaccines. Coming to terms with fuzzy recognition sites and ill-defined structure-function relationships in immunology. *Vaccine* (1999) **18**:216–21. doi:10.1016/S0264-410X(99)00192-9
93. Kuntz ID. Structure-based strategies for drug design and discovery. *Science* (1992) **257**:1078–82. doi:10.1126/science.257.5073.1078
94. Van Regenmortel MHV. The rational design of biological complexity: a deceptive metaphor. *Proteomics* (2007) **7**:965–75. doi:10.1002/pmic.200600407
95. Schiffner T, Sattentau QJ, Dorrell L. Development of prophylactic vaccines against HIV-1. *Retrovirology* (2013) **10**:72. doi:10.1186/1742-4690-10-72
96. Salmon WC. *Causality and Explanation*. Oxford: Oxford University Press (1998).
97. Lipton P. What good is an explanation? In: Cornwell J, editor. *Explanations. Styles of Explanation in Science*. Oxford: Oxford University Press (2004). p. 1–21.
98. Dupré J. *The Disorder of Things. Metaphysical Foundations of the Disunity of Science*. Cambridge, MA: Harvard University Press (1993).
99. Gannon F. Too complex to comprehend? *EMBO Rep* (2007) **8**:705. doi:10.1038/sj.embor.7401041
100. Diskin R, Scheid JF, Marcovecchio PM, West AP Jr, Klein F, Gao H, et al. Increasing the potency and breadth of an HIV antibody by using structure-based rational design. *Science* (2011) **334**:1289–93. doi:10.1126/science.1213782
101. Gunawardena J. Models in biology: 'accurate descriptions of our pathetic thinking'. *BMC Biol* (2014) **12**:29. doi:10.1186/1741-7007-12-29
102. Ylikoski P. The illusion of depth of understanding in science. In: De Regt HW, Leonelli S, Eigner K, editors. *Scientific Understanding*. Pittsburgh: University of Pittsburgh Press (2009). p. 100–19.
103. Van Fraassen BC. Experience. *The Empirical Stance*. New Haven: Yale University Press (2002). p. 111–52.
104. Hacking Ian. *Representing and Intervening. Introductory Topics in the Philosophy of Natural Science*. Cambridge, UK: Cambridge University Press (1983). 287 p.
105. Leonelli S. Understanding in biology: the impure nature of biological knowledge. In: De Regt HW, Leonelli S, Eigner K, editors. *Scientific Understanding*. Pittsburgh: University of Pittsburgh Press (2009). p. 189–209.
106. Kelley L, Scott M. The evolution of biology. *EMBO Rep* (2008) **9**:1163–7. doi:10.1038/embor.2008.212
107. Sok D, Doores KJ, Briney B, Le KM, Saye-Francisco KL, Ramos A, et al. Promiscuous glycan site recognition by antibodies to the high-mannose patch of gp120 broadens neutralization of HIV. *Sci Transl Med* (2014) **6**:236ra63. doi:10.1126/scitranslmed.3008104
108. Alberts B, Kirschner MW, Tilghman S, Varmus H. Rescuing US biomedical research from its systemic flaws. *Proc Natl Acad Sci U S A* (2014) **111**:5773–7. doi:10.1073/pnas.1404402111

Conflict of Interest Statement: The author declares that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Received: 26 July 2014; accepted: 05 November 2014; published online: 18 November 2014.
Citation: Van Regenmortel MHV (2014) An outdated notion of antibody specificity is one of the major detrimental assumptions of the structure-based reverse vaccinology paradigm, which prevented it from helping to develop an effective HIV-1 vaccine. Front. Immunol. 5:593. doi: 10.3389/fimmu.2014.00593
This article was submitted to HIV and AIDS, a section of the journal Frontiers in Immunology.

frontiers in
IMMUNOLOGY

ORIGINAL RESEARCH ARTICLE
published: 28 August 2014
doi: 10.3389/fimmu.2014.00398

The antibody germline/maturation hypothesis, elicitation of broadly neutralizing antibodies against HIV-1 and cord blood IgM repertoires

***Ponraj Prabakaran*[1,2]*, *Weizao Chen*[1] *and Dimiter S. Dimitrov*[1]**

[1] *Protein Interactions Group, Laboratory of Experimental Immunology, Cancer and Inflammation Program, Center for Cancer Research, National Cancer Institute, National Institutes of Health, Frederick, MD, USA*
[2] *Basic Science Program, Leidos Biomedical Research, Inc., Frederick, MD, USA*

Edited by:
Marc H. V. Van Regenmortel, University of Strasbourg, France

Reviewed by:
Leonidas Stamatatos, Seattle Biomedical Research Institute, USA
Neil S. Greenspan, Case Western Reserve University, USA

***Correspondence:**
Ponraj Prabakaran, Protein Interactions Group, Laboratory of Experimental Immunology, Cancer and Inflammation Program, National Cancer Institute, National Institutes of Health (NIH), Building 576, Room 103, Frederick, MD 21702, USA
e-mail: prabakaran.ponraj@nih.gov

We have previously observed that all known potent broadly neutralizing antibodies (bnAbs) against HIV-1 are highly divergent from their putative germline predecessors in contrast to bnAbs against viruses causing acute infections such as henipaviruses and SARS CoV, which are much less divergent from their germline counterparts. Consequently, we have hypothesized that germline antibodies may not bind to the HIV-1 envelope glycoprotein (Env) because they are so different compared to the highly somatically mutated HIV-1-specific bnAbs. We have further hypothesized that the immunogenicity of highly conserved epitopes on the HIV-1 envelope glycoproteins (Envs) may be reduced or eliminated by their very weak or absent interactions with germline antibodies and immune responses leading to the elicitation of bnAbs may not be initiated and/or sustained. Even if such responses are initiated, the maturation pathways are so extraordinarily complex that prolonged periods of time may be required for elicitation of bnAbs with defined unique sequences. We provided the initial evidence supporting this antibody germline/maturation hypothesis, which prompted a number of studies to design vaccine immunogens that could bind putative germline predecessors of known bnAbs and to explore complex B cell lineages. However, guiding the immune system through the exceptionally complex antibody maturation pathways to elicit known bnAbs remains a major challenge. Here, we discuss studies exploring the antibody germline/maturation hypothesis as related to elicitation of bnAbs against HIV-1 and present our recent data demonstrating the existence of germline-like precursors of VRC01 antibodies in a human cord blood IgM library.

Keywords: HIV-1, vaccine, broadly neutralizing antibody, cord blood IgM, germline antibody, 454 sequencing

INTRODUCTION

Elicitation of broadly neutralizing antibodies (bnAbs) targeting the HIV-1 envelope glycoproteins (Envs), the key to an effective HIV-1 vaccine, remains elusive. Previous studies have demonstrated several properties of the HIV-1 Envs that could limit their ability to elicit bnAbs. These include protection of the conserved structures by variable loops (1–3), remarkable genetic diversity (4), a glycan shield (5), steric occlusion (6), and conformational masking (7). Until 6 years ago, only a handful of bnAbs, including b12, 2G12, 2F5, and 4E10, were known. Although the structural and functional studies of those bnAbs revealed some important neutralization epitopes (8), such bnAbs have not been successfully elicited by any vaccination approach.

In 2007, we first noted that in HIV-1 specific bnAbs the number of amino acid mutations from their closest corresponding germline sequences was significantly higher than that of bnAbs against the SARS CoV coronavirus, and Hendra and Nipah viruses, which cause self-limiting acute infections (9). Using a large non-immune IgM library, we identified several HIV-1 Env specific antibodies and found that they had fewer somatic mutations than the HIV-1 bnAbs, as well as limited neutralizing activity (9). These findings indicated that elicitation of HIV-1 bnAbs would require far more extensive maturation processes than those needed to generate the bnAbs against the SARS CoV and henipaviruses. So, we have suggested that the difficulty of eliciting these bnAbs may be due, at least in part, to the complex and prolonged maturation pathways required for the development of bnAbs against the HIV-1, which can take long time (10). We thus speculated that this may represent another significant challenge in the development of effective HIV-1 vaccines.

We quantified the number of mutations in human monoclonal antibodies (mAbs) that we selected from phage libraries generated from an HIV-1-infected patient with a known time of infection (10). We calculated the number of amino acid mutations per heavy chain V gene, and defined it as antibody somatic mutational diversification (ASMD). We compared the extent and dynamics of the ASMD between HIV-1-specific mAbs and a panel of SARS CoV-specific mAbs. Our experiments based on the ASMD predicted that elicitation of HIV-1-specific bnAbs would take at least 3 years. An illustrative mathematical model using the ASMD rate based on

an exponential time dependent function suggested that a much longer time would be needed for the required maturation, unless somatic diversification had already been initiated from an intermediate antibody. Thus, all these initial studies corroborated our hypothesis that the infrequent occurrence or absence of bnAbs in HIV-1-infected patients could be due, at least in part, to the lack or limited availability of B cell receptors that rapidly mature into bnAbs. Therefore, we suggested that appropriate immunization protocols of long duration need to be developed using the knowledge gained from the exploration of antibody maturation pathways in humans (10).

From the striking observation that all known potent HIV-1 bnAbs are highly divergent from their putative germline predecessors in contrast to bnAbs against henipaviruses and SARS CoV coronavirus, we hypothesized that, since the germline antibodies are so different compared to the highly somatically mutated HIV-1 bnAbs, they may not bind to the Env. This led us to the hypothesis that the immunogenicity of the highly conserved epitopes on the HIV-1 native envelope glycoproteins (Envs) is reduced or eliminated by their very weak or absent interactions with germline antibodies, which could not initiate and/or sustain immune responses leading to elicitation of bnAbs: even if immune responses are initiated and sustained, the maturation pathways are so complex that help and long times may be needed for their elicitation. To test our antibody germline/maturation hypothesis, we designed germline-like antibodies corresponding to the known bnAbs b12, 2G12, 2F5, X5, m44, and m46 (the latter three antibodies were discovered in our laboratory and possess HIV-1 cross-reactivity with moderate neutralizing activities) and evaluated them for binding to Envs (11). We found that while germline-like X5, m44, and m46 bound to Envs with relatively high affinity, the germline-like precursors of b12, 2G12, and 2F5 failed to bind Envs in an ELISA assay although their corresponding mature bnAbs bound strongly. These results provided initial evidence that the Env structures containing conserved epitopes might not initiate humoral responses due to limited or absent binding to the germline precursors of bnAbs. These germline precursors may also be of limited availability as recently reported (12).

Following that initial study, we expanded our investigation to different variants of the two different antibodies (b12 and X5) including their closest germline counterparts and several germline-like intermediates (13). The experiments showed that b12 intermediate antibodies neutralized only some HIV-1 isolates with relatively weak potency. In contrast, intermediates of X5 neutralized a subset of the tested HIV-1 isolates with efficiencies comparable to those of the matured X5. These results helped explain the relatively high immunogenicity of the coreceptor binding site on gp120 and the abundance of CD4-induced (CD4i) antibodies in HIV-1-infected patients (X5 is a CD4i antibody) as well as the maturation pathway of X5. In the case of b12, germline-like intermediates along the maturation pathway were shown to not only bind some Envs but also human self antigens, suggesting that antigens other than the Envs could help guide the immune system through the b12 maturation pathway.

Therefore, we proposed a conceptually new vaccination approach, in which it is critical to identify primary immunogens that bind to the germline antibodies that are predecessors of bnAbs. If needed, these immunogens should be combined with secondary immunogens that recognize intermediate and/or matured antibodies to guide the immune system through the prolonged, complex maturation pathways (14). In this respect, we envisioned that the knowledge of human antibodyomes would become indispensable to elucidate the origin, diversity, and maturation pathways of bnAbs and discover germline-like intermediates of bnAbs that could provide a basis for the design of novel HIV-1 vaccine immunogens (14, 15).

In recent years, several groups have reported a number of new bnAbs that were identified from multiple HIV-1 infected individuals using designed novel antigen baits and advanced technologies implemented in isolating human mAbs and high-throughput sequencing (16). Particularly, Haynes, Kwong, Stamatatos, Scheif et al. have dealt with a large amount of data delineating structural, genetic determinants, and maturation pathways of different bnAbs. These studies not only confirmed our previous findings that the Envs fail to engage germline versions of bnAbs but also suggested possible holes in B cell repertoires and demonstrated the implications of our antibody germline/maturation hypothesis for finding germline-like precursors, intermediates as well as for designing immunogens that could potentially bind to such bnAb intermediates. In this report, we discuss the recent advancements in HIV-1 vaccine research in the context of the antibody germline/maturation hypothesis, and highlight critical factors to be considered when exploring germline-like precursors and intermediates of bnAbs. We also report for the first time using 454 sequencing data analysis of a human cord blood IgM library to identify putative germline precursors of the heavy and light chains of VRC01-like antibodies. These naturally occurring cord blood-derived VRC01-like heavy and light chains may be useful as putative templates for designing novel vaccine immunogens that can lead to the elicitation of VRC01-like antibodies and for understanding the maturation pathways of this bnAb. Still there are major challenges to be overcome. New empirical and semi-empirical approaches could be successful; recently, new paradigms were discussed that could better fit our increased knowledge of HIV immunopathology and which could possibly be more helpful in guiding future vaccine research than did past unsuccessful approaches (17).

MATERIALS AND METHODS

ANTIBODYOME DATABASE AND TOOLS

DNA isolation, amplification, and 454 sequencing of the human cord blood IgM library were previously described in detail (18, 19). For quality control, we trimmed the 454 sequence reads and retained only sequences with lengths of more than 300 nucleotides, covering the entire antibody variable domains consisting of all three complementarity determining regions (CDRs) along with framework regions (FRs). We used IMGT/HighV-QUEST for immunogenetic and statistical analyses (20). The output results from the IMGT/HighV-QUEST analysis were stored in a local PostgreSQL database, and structured query language (SQL) was used to retrieve the data for further analysis. Statistical calculations were carried out using JMP10® statistical software (SAS Institute, Cary, NC, USA).

COMPUTATIONAL ANALYSIS OF ANTIBODY SEQUENCES

Antibody sequences from IGHV1-2 and IGK3-11 lineages were retrieved from our local antibodyome database consisting of immunogenetic data derived from 454 sequencing of the human cord blood IgM library using SQL statements. Amino acid sequence identities between each of the selected lineage sequences from the 454 sequence data and pertinent germline sequences were calculated based on the pairwise alignment using local BLAST as implemented in BioEdit v7.0.9 (21). Phylogenetic analysis was carried out using the Archaeopteryx software (22).

RESULTS AND DISCUSSION

EXPLORING THE ANTIBODY GERMLINE/MATURATION HYPOTHESIS

Our earlier observation of the extensive maturation of HIV-1 bnAbs in contrast to those against some viruses causing acute infections led to the antibody germline/maturation hypothesis (9–11, 13, 14). According to this hypothesis, it is critical to identify immunogens that would bind to germline and/or intermediate antibodies of bnAbs, as well as the exploration of antibodyomes could be useful for identifying such immunogens (14). **Figure 1** describes the timeline involving some of the key developments in current HIV-1 vaccine research focused on antibody germline-like intermediates and maturation pathways of bnAbs. Major research efforts in this direction were spearheaded by deep sequencing and structural biology studies of VRC01-like and other CD4-binding site (CD4b) antibodies from HIV-1-infected individuals. These studies delineated possible maturation pathways of such antibodies with high levels of somatic mutations and convergence in antibody recognition (23, 24). Both studies revealed that the putative germline precursors of these antibodies had weak or no apparent affinity for Env, and acquisition of a large number of somatic mutations were needed for the breadth and potency of these antibodies. These studies also explored antibody diversity and found many intermediates of similar lineages of the heavy chain genes from the two IGHV families VH1-2 and VH1-46 that paired with different light chain genes. Thus, analysis of the VRC01-related antibodyome from HIV-1 infected patients revealed B cell maturation pathways that may help guide the vaccine-induced elicitation of such antibodies. However, if we could find germline-like intermediates of such bnAbs from a naïve antibody repertoire, then

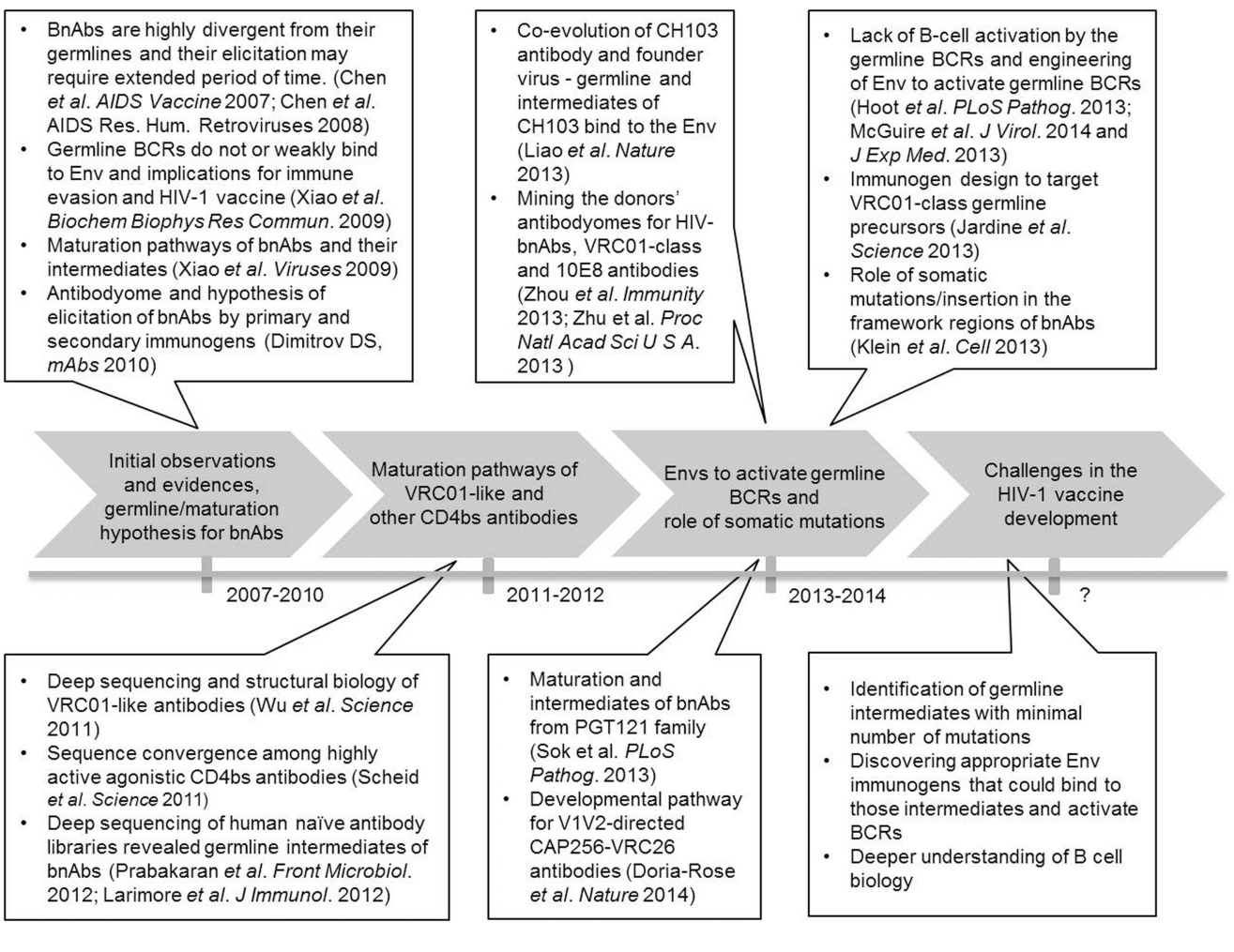


FIGURE 1 | Timeline of the antibody germline/maturation hypothesis and experiments.

potential vaccine immunogens developed based on those templates would stimulate an adequate B cell immune response in healthy humans. To this end, we identified VRC01-like intermediate antibodies from a naïve antibody library of human cord blood, which is presented later in the text.

We previously analyzed the IgM repertoires of healthy individuals and identified several intermediates of b12 from the VH1-3 gene family (15). Sequence analysis of 28,925 unique sequences from the IgM repertoires revealed a CDRH3 with a length (20 amino acids) and sequence similar (50%) to that of the b12 CDRH3, but the V gene associated with that CDRH3 was found to be HV4-b (15). This finding indicates that long CDRH3s may not be a limiting factor for the development of bnAbs (25) although long CDRH3 motifs with certain amino acid preferences and/or associations with particular heavy or light chain families favoring polyreactivity may not be undermined.

Stamatatos and coworkers have conducted experiments screening a large panel of recombinant Envs for binding to the germline predecessors of b12, NIH45-46, and 3BNC60 to test how Env immunogens interact with the predicted germline versions of known bnAbs (26). They found that the mature bnAbs reacted with diverse Envs but the corresponding germline antibodies did not. They examined in detail the germline b12 and its chimeric forms – either the germline heavy chain paired with the mature light chain and vice versa – to test whether they could interact with any of the recombinant Envs derived from clade A, B, and C viruses. Among all the recombinant Envs tested, at least one Env (QH0692) was found to bind a b12 chimera with a mature heavy chain. However, this chimera failed to mediate calcium mobilization, indicating no BCR activation via BCR-antigen engagement. In other studies, they found that the elimination of certain conserved glycosylation sites on Envs led to the binding of germline versions of VRC01 and NIH45-46 and BCR activation (27) but that the modified Envs did not interact with PG9 and 447-52D germlines (28).

Haynes and coworkers have succeeded in finding Envs capable of engaging the germline versions of a CD4bs bnAb, CH103, while studying the co-evolution of the antibody in an HIV-1 infected patient (29). They found that CH103 is less mutated than most other CD4bs bnAbs, and importantly that the unmutated common ancestor of the CH103 lineage avidly bound the transmitted/founder HIV-1 Envs. This finding suggests that early founder Envs could bind optimally to the germline and intermediate versions of CH103, and therefore, are promising vaccine immunogens, representing an important step forward in HIV-1 vaccine development.

Similarly, the maturation pathway of the potent V1V2-directed HIV-neutralizing antibody, CAP256–VRC26, has been described, in which a germline-like intermediate with a 35-amino acid residue long CDRH3 was shown to bind and neutralize the superinfecting virus weakly, but did not bind or neutralize heterologous viruses (30). These results suggest that the CAP256–VRC26 lineage could be initiated by using a rare superinfecting-virus-like V1V2 Env.

In another successful effort in identifying an Env that could engage the germline versions of bnAbs, Scheif and coworkers devised a computation-guided approach combined with *in vitro* screening to engineer a gp120 outer domain. The designed protein not only bound to multiple VRC01-class bnAbs and their germline precursors but also activated B cells expressing diverse intermediates of the bnAbs (31). Therefore, priming with the protein and subsequent boosting with more native immunogens could help induce early somatic mutations and the ultimate elicitation of VRC01-class bnAbs.

Interestingly, Nussenzweig and coworkers' study showed that somatic mutations of the FRs and insertions of some bnAbs are required for their broad and potent HIV-1 neutralizing activity (32). Based on structural information, they made different germline versions of VRC01, NIH45–46, 12A21, and 3BNC117, and found that mutations in FRs were also essential for binding, breadth, and potency of most bnAbs. This suggested that certain framework mutations could be critical and should be preserved for designing the intermediates of such bnAbs. Several other studies mining the HIV-1 infected donors' antibodyomes (33–35) revealed putative intermediates of bnAbs. Many of them with lower levels of somatic hyper mutations could bind to selective Envs; for example, intermediates of PGT121-134 were able to preferentially bind native Envs relative to monomeric gp120 (36). We also identified 2F5-like antibodies (m66 and m66.6) with much fewer mutations than 2F5 and suggested their use as a model system for elicitation of such antibodies (37, 38).

All these newly discovered bnAbs raise the hopes for effective HIV-1 vaccine development as they reveal characteristic features of bnAbs that could help us understand the immunological basis critical for their production and also serve as templates for rational vaccine design. Therefore, the focus has been dramatically shifted to explore and overcome the immunological hurdles associated with the elicitation of bnAbs, namely, extensive somatic mutations of bnAbs. Major challenges remain in identification of intermediates with a minimal number of mutations, and appropriate Env immunogens that would bind such intermediates and activate BCRs, which can lead to the maturation of the intermediate antibodies to bnAbs. Recently, new paradigms that better fit our increased knowledge of HIV immunopathology and which may be more helpful in guiding future vaccine research than did past unsuccessful approaches were discussed (17).

IDENTIFICATION OF PUTATIVE GERMLINE-LIKE INTERMEDIATES IN THE MATURATION PATHWAYS OF VRC01

We previously characterized the human cord blood cell-derived IgM antibodies using 454 sequencing to study gene diversity and somatic mutations (19). Naïve germline antibody repertoires, particularly from babies, may be quite unique for understanding the B cell maturation pathways, as they can also mount an immune response against HIV-1 as recently found (39). Our earlier gene usage analysis of the cord blood IgM repertoire showed the biased IGHV gene usages (19) as similar to adult IgM repertoires (40). However, we already noted that the IGHV1-2 gene usage was significantly higher in the cord blood IgM repertoire, i.e., an overall contribution of 20% as compared to 8% in adult IgM repertoires. This suggested that the cord blood IgM repertoire may be advantageous for the exploration of the IGHV1-2*02 lineages when studying germline precursors and intermediates of VRC01 heavy chain. A total of 5,624 heavy chain and 1,096 light chain sequences of IGHV1-2 and IGKV3-11 lineages, respectively, were used to

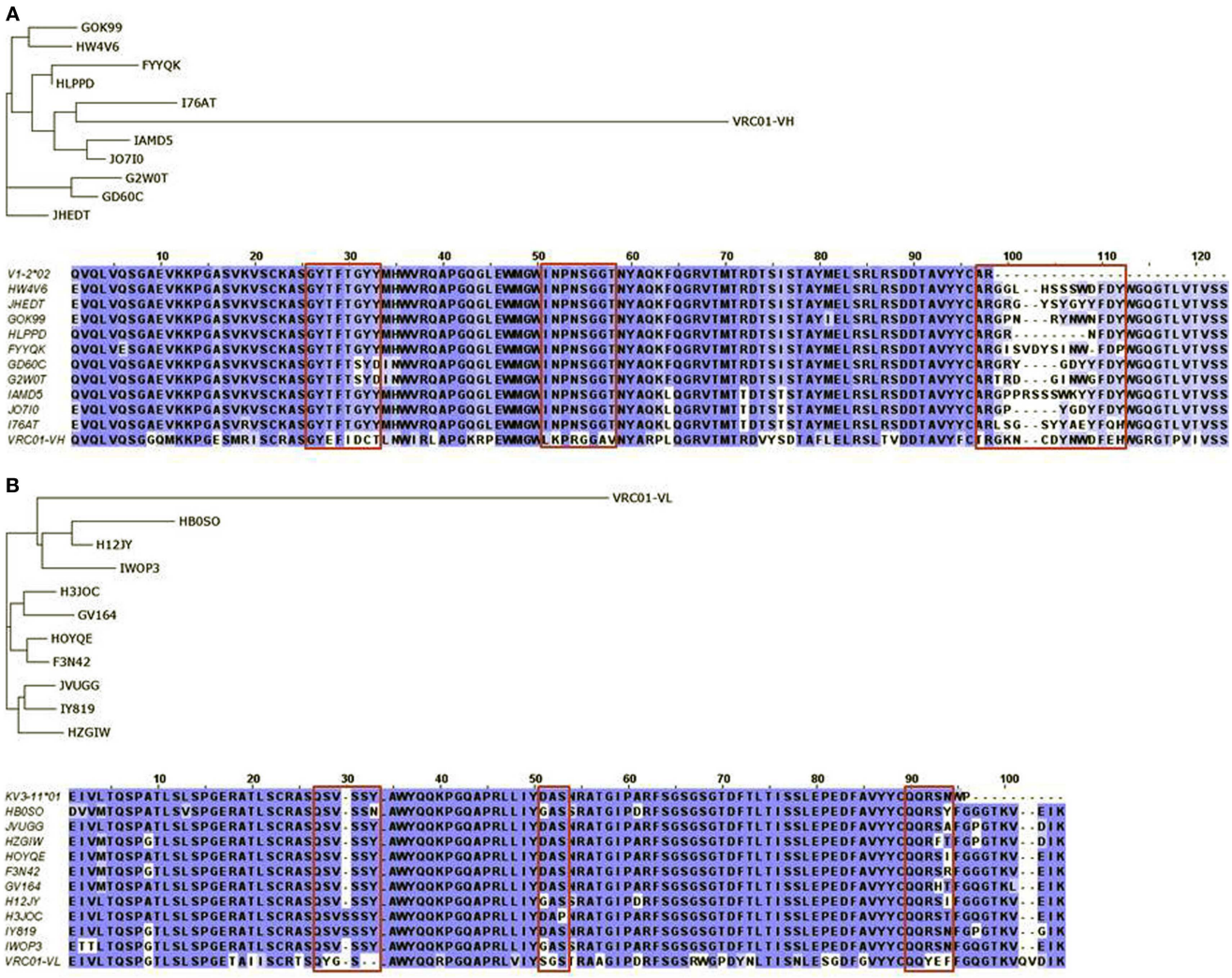


FIGURE 2 | Phylogram and sequence alignment of selected (A) heavy and (B) light chain sequences found in human cord blood IgM libraries of IGHV1-2 and IGKV3-11 lineages, respectively. Phylogenetic trees constructed using the amino acid sequences of top 10 putative germline-like precursors as found in the cord blood library along with mature heavy and light chains of VRC01 are shown. Multiple sequence alignments of heavy and light chain sequences along with their corresponding germline sequences, V1-2*02 and KV3-11*01, made using BLOSUM62 scoring matrices, conserved substitution (blue) and non-conserved substitution (white) are shown.

select the top 10 sequences as closest intermediates for VRC01 in each heavy and light chain categories by using local BLAST searching. We performed phylogenetic analysis of the selected sequences to identify genetic relationships among VRC01-like intermediates of heavy (**Figure 2A**) and light (**Figure 2B**) chains. We found two of the antibody heavy chains, HWAV6 and JHEDT, which were 100% identical to the IGHV1-2*02 germline sequence. Remarkably, their CDRH3 sequences had the same length (14 amino acids) as that of the VRC01 heavy chain. For these 10 heavy chain sequences, the CDRH3 lengths ranged from 8 to 16 amino acids with sequence variations at the junctions. One of the germline sequences, JHEDT, had a point mutation at Cys100Tyr (Kabat numbering) of CDRH3 that exactly mimicked the residue Tyr100 of CDRH3 in VRC01. The residue Tyr100 at CDRH3 of VRC01 is most likely contributed by the IGHD3-16*02 germline with a point mutation Cys100Tyr. The other heavy chain sequence I76AT, which was the closest to VRC01 heavy chain, also had the same mutation at Cys100Tyr. One of the other germline sequences, HWAV6, had Trp100B (Kabat numbering) of CDRH3 that exactly mimicked the residue Trp100B of CDRH3 in VRC01. Intriguingly, the Trp100B residue is a junctional amino acid of the CDRH3 in germline HWAV6, and it exactly replicates the Trp100B junctional residue of CDRH3 in VRC01. This suggests a possible maturation mechanism involved in the VRC01-like intermediates where junctional amino acids could determine the maturation pathway far preceding the somatic hypermutation required for affinity maturation (41). Most of the closest IGHV genes, 8 out of 10 shown in the **Figure 2A**, have at least one mutation in the V region, and

two sequences, G2W0T and GD60C, have two mutations at each of the CDRH1. The pre-existing amino acid mutations found in the V region and CDRH3 sequence information may inform the design of heavy chain germline-like precursors and intermediates, and help naturally reconstruct the B cell clonal lineages in the maturation pathways of VRC01.

Light chain recognition of Envs by VRC01 and VRC01-related antibodies has been studied in detail using structural and 454 sequencing data (33). The VRC01 light chain commonly uses the IGKV1-33 lineage and has a characteristic five amino acid long CDRL3 and a distinctive two amino acid deletion in CDR L1. Therefore, we selected the IGKV1-33 lineage sequences with five amino acid length CDRL3s, but no sequences were found with a two amino acid deletion in CDR L1 (**Figure 2B**). All of them had either framework or CDR mutations or both. Four of them had a point mutation at CDRL1 and seven of them had a point mutation at CDRL3.

The structural basis for germline gene usage of VRC01-related antibodies targeting the CD4bs has been previously described (42), which revealed a set of signature features for these antibodies that were verified by mutagenesis. These signature features explained the origin of the IGVH1-2 gene and antibody resistance for some Env sequences. We found that characteristic residues including the Trp100B of heavy chains were conserved while light chains did not have any characteristic residues as reported previously (42). However, other pre-existing amino acid mutations in light chains could have implications for the VRC01-related intermediates with a characteristic CDRL3 of five amino acid length.

DISTRIBUTIONS OF CDR LENGTHS AND AMINO ACIDS IN THE VRC01-RELATED GERMLINE GENES

We analyzed the amino acid length distributions of CDRH3 and CDRL3 sequences that were of VRC01 origins, namely, IGHV1-2 and IGKV3-11 for heavy and light chains, respectively, as derived from the human cord blood IgM library (**Figure 3**). The CDRH3 lengths ranged from 4 to 27 amino acids, indicating high CDR3 length diversity (**Figure 3A**). VRC01 has a CDRH3 length of 14 amino acids, which is shorter than those of most other anti-HIV-1 antibodies (25). The LCDR3 lengths ranged from 4 to 14 amino acids (**Figure 3B**). The CDRL3 of VRC01 has a characteristic length of five amino acids with a mature genetic signature (33). Analysis of the human cord blood IgM repertoire showed only a fraction of such light chains with a shorter length of five amino acids (**Figure 3B**). Frequency distributions of amino acid compositions of CDRH1 and CDRH2 in IGHV1-2 sequences as observed in the human cord blood IgM repertoire are plotted in **Figures 3C,D**,

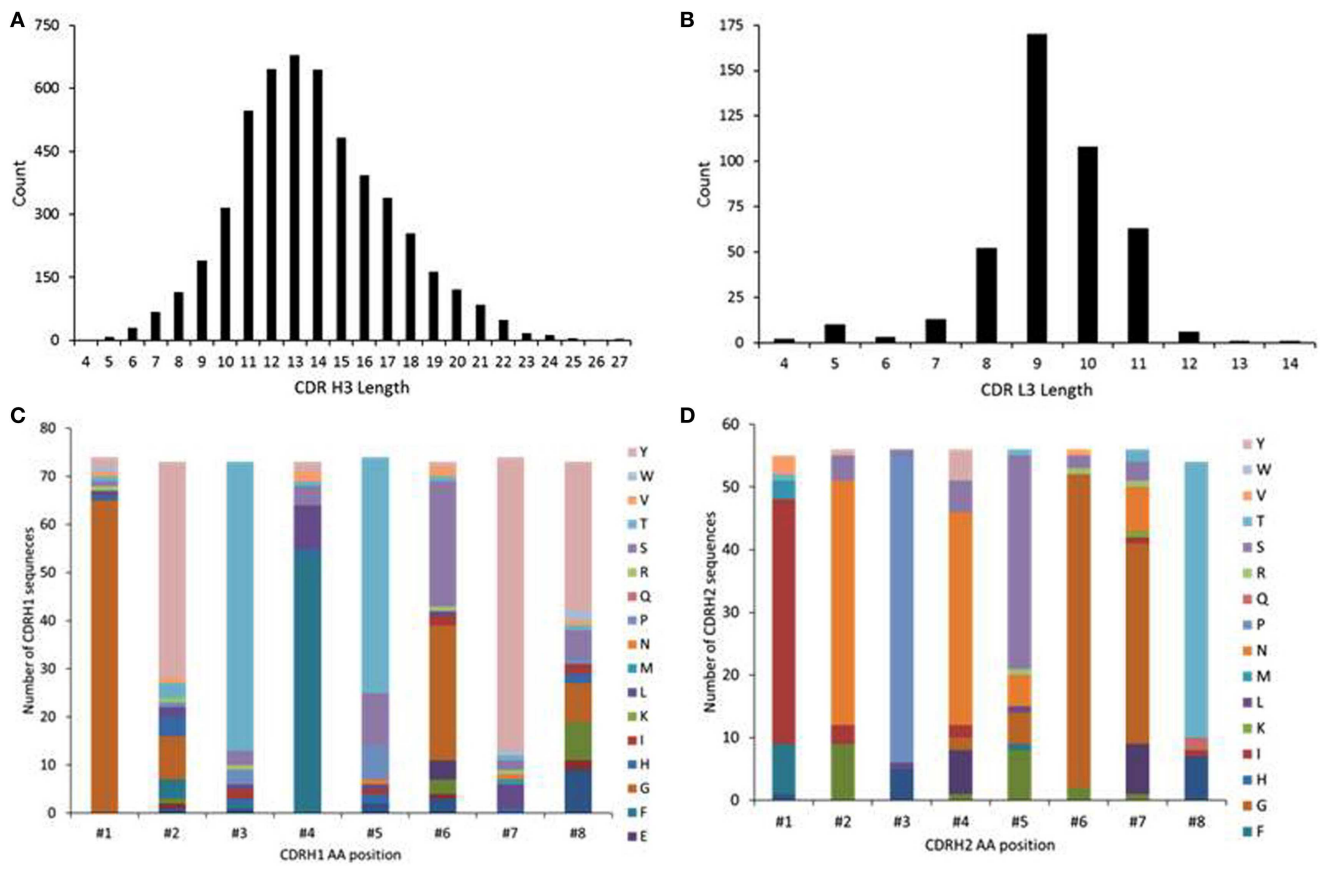


FIGURE 3 | Distributions of amino acid length in (A) CDRH3, (B) CDRL3 and amino acid compositions in (C) CDRH1 and (D) CDRH2 as derived using the gene families of IGHV1-2 and IGKV3-11 in human cord blood IgM library showing the CDR3 lengths diversity and pre-existing mutations at CDRH1 and H2.

respectively. These plots show that there are position specific variations in the CDRH1 and CDRH2 regions of IGHV1-2 genes. These could indicate possible IGHV1-2 specific pre-existing amino acid mutations in CDRH1 and CDRH2, as observed in several naïve antibody heavy chain sequences, which could inform the design of germline precursors and intermediates of VRC01-like antibodies.

V–D–J RECOMBINATION DIVERSITY AND IGHD READING FRAME USAGES IN THE VRC01-RELATED GERMLINE GENES

We previously observed that the V–D–J rearrangement patterns occurred at different frequencies with 1,430 V–D–J combinations in a human cord blood IgM repertoire (19). **Figure 4A** shows the V–D–J diversity associated with IGHV1-2 gene sequences using a bubble plot for comparison with different D and J genes. The VRC01 heavy chain uses IGHD3-16 and IGHJ2 genes to recombine with IGHV1-2. However, other VRC01-related antibodies exhibit a skewed usage of IGHJ genes although at least three different IGHJ genes (IGHJ1, IGHJ2, and IGHJ4) are involved (23). As the human cord blood IgM library has a large functional V–D–J diversity, it can be used to identify potential VRC01-like heavy chain germline precursors and intermediates.

In jawed vertebrates the expressed heavy chains may use any of the six IGHD reading frames (RFs); however, RF1 is thought to be the preferred one as it mostly encodes tyrosine and glycine. The remaining five RFs encode either hydrophobic or charged amino acids, but the use of inverted RF1, RF2, and RF3 are discouraged. Preferential usage of IGHD RFs has been long implicated in B cell development and antigen-specific antibody production (43–45), and selected based upon its amino acid content (46). Genetic control of IGDH RF preference over the regulation of repertoire development has been recognized (47). Here, we have analyzed the productive IGHD RF usages in a human cord blood IgM library. Frequency distribution of RFs is plotted using a pie chart as depicted in **Figure 4B**. We noted that there were not any highly restricted usages of the IGHD RFs although some preferential usages depending on the IGHD genes were found. This clearly indicates that IGHD RFs diversity could add more diverse amino acid contents leading to enormous CDRH3 diversity. It may also be possible that intermediates with different RF choices play a critical role in selecting certain maturation pathways efficiently.

CONCLUSION

The antibody germline/maturation hypothesis led to a paradigm shift in the design of immunogens for bnAb elicitation, as well as the realization of the importance of the complexity of the bnAb maturation pathways, and exploration of human antibodyomes (14). In fact, human antibodyome exploration is also promising for other fields of science and medicine (14, 48). This antibodyome approach is now a major direction of research in the HIV-1 vaccine field (16, 49). An important goal is to precisely identify naturally occurring germline-like precursors and intermediates of bnAbs that could help designing novel immunogens, which could activate the corresponding BCRs and drive the immune system to produce bnAbs within a short period of time. We presented an approach using a human cord blood IgM library to identify putative germline precursors and intermediates of VRC01-like heavy and light chains, which could be useful in reconstructing the B cell clonal lineages in the maturation pathways of VRC01-related bnAbs. This method has the potential to help in the identification of naturally occurring germline-like precursors and intermediates of any known bnAb and in the development immunogens based on HIV-1 Envs (50) and peptides (51), as well as non-HIV-1 molecules (12). However, major challenges remain and new paradigms that better fit our increased knowledge of HIV immunopathology could possibly be more helpful in guiding future vaccine research than did past unsuccessful approaches (17).

ACKNOWLEDGMENTS

We thank the Laboratory of Molecular Technology of SAIC-Frederick, Inc., for providing Roche 454 sequencing service. We thank Tina Ju for critically reading the manuscript. This research was supported by the Intramural Research Program of the NIH, National Cancer Institute, Center for Cancer Research, the Intramural AIDS Targeted Antiviral Program (IATAP) of the NIH and by Federal funds from the NIH, National Cancer Institute, under Contract Nos. NO1-CO-12400 and HHSN261200800001E. The

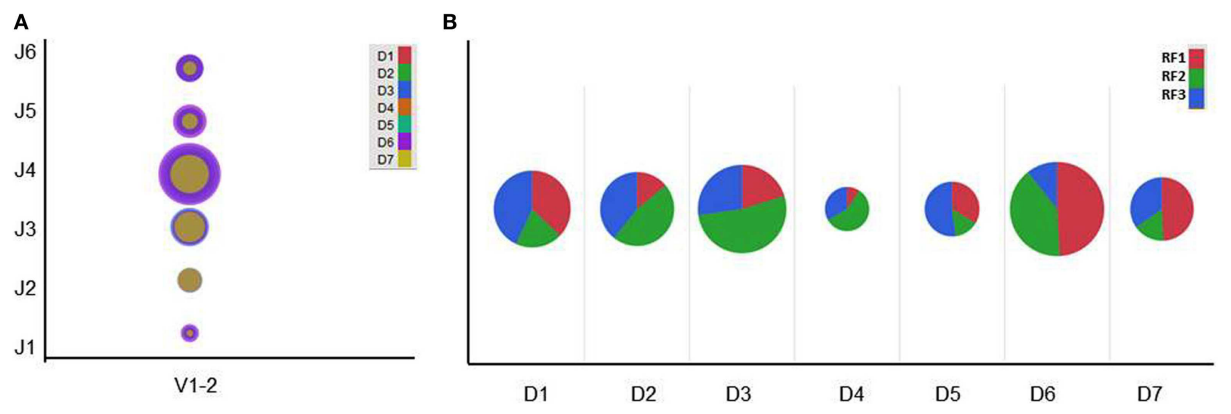


FIGURE 4 | (A) Bubble plot showing the V–D–J diversity associated with IGHV1-2 gene sequences and **(B)** Pie chart showing the IGHD reading frame (RF) usages in human cord blood IgM library.

content of this publication does not necessarily reflect the views or policies of the Department of Health and Human Services, nor does the mention of trade names, commercial products, or organizations imply endorsement by the U.S. Government.

REFERENCES

1. Wyatt R, Kwong PD, Desjardins E, Sweet RW, Robinson J, Hendrickson WA, et al. The antigenic structure of the HIV gp120 envelope glycoprotein. *Nature* (1998) **393**:705–11. doi:10.1038/31514
2. Julien JP, Cupo A, Sok D, Stanfield RL, Lyumkis D, Deller MC, et al. Crystal structure of a soluble cleaved HIV-1 envelope trimer. *Science* (2013) **342**:1477–83. doi:10.1126/science.1245625
3. Lyumkis D, Julien JP, de Val N, Cupo A, Potter CS, Klasse PJ, et al. Cryo-EM structure of a fully glycosylated soluble cleaved HIV-1 envelope trimer. *Science* (2013) **342**:1484–90. doi:10.1126/science.1245627
4. McBurney SP, Ross TM. Viral sequence diversity: challenges for AIDS vaccine designs. *Expert Rev Vaccines* (2008) **7**:1405–17. doi:10.1586/14760584.7.9.1405
5. Wei X, Decker JM, Wang S, Hui H, Kappes JC, Wu X, et al. Antibody neutralization and escape by HIV-1. *Nature* (2003) **422**:307–12. doi:10.1038/nature01470
6. Klein JS, Gnanapragasam PN, Galimidi RP, Foglesong CP, West AP Jr, Bjorkman PJ. Examination of the contributions of size and avidity to the neutralization mechanisms of the anti-HIV antibodies b12 and 4E10. *Proc Natl Acad Sci U S A* (2009) **106**:7385–90. doi:10.1073/pnas.0811427106
7. Kwong PD, Doyle ML, Casper DJ, Cicala C, Leavitt SA, Majeed S, et al. HIV-1 evades antibody-mediated neutralization through conformational masking of receptor-binding sites. *Nature* (2002) **420**:678–82. doi:10.1038/nature01188
8. Burton DR, Desrosiers RC, Doms RW, Koff WC, Kwong PD, Moore JP, et al. HIV vaccine design and the neutralizing antibody problem. *Nat Immunol* (2004) **5**:233–6. doi:10.1038/ni0304-233
9. Chen W, Zhu Z, Zhang M, Prabakaran P, Feng Y, Quinnan GV, et al. Extensive maturation of cross-reactive HIV-1 neutralizing antibodies but not of neutralizing antibodies against the SARS CoV, Nipah and Hendra viruses. *AIDS Vaccine* (2007) **P10-18**:142. doi:10.1038/nature12053
10. Chen W, Zhu Z, Zhang MY, Macagno A, Prabakaran P, Owens J, et al. All known cross reactive HIV-1 neutralizing antibodies are highly divergent from germline and their elicitation may require prolonged periods of time. *AIDS Res Hum Retroviruses* (2008) **24**:11–2.
11. Xiao X, Chen W, Feng Y, Zhu Z, Prabakaran P, Wang Y, et al. Germline-like predecessors of broadly neutralizing antibodies lack measurable binding to HIV-1 envelope glycoproteins: implications for evasion of immune responses and design of vaccine immunogens. *Biochem Biophys Res Commun* (2009) **390**:404–9. doi:10.1016/j.bbrc.2009.09.029
12. Yang Z, Li J, Liu Q, Yuan T, Zhang Y, Chen LQ, et al. Identification of non-HIV immunogens that bind to germline b12 predecessors and elicit cross-reactive neutralizing HIV-1 antibodies. *AIDS* (2014) (in press).
13. Xiao X, Chen W, Feng Y, Dimitrov DS. Maturation pathways of cross-reactive HIV-1 neutralizing antibodies. *Viruses* (2009) **1**:802–17. doi:10.3390/v1030802
14. Dimitrov DS. Therapeutic antibodies, vaccines and antibodyomes. *MAbs* (2010) **2**:347–56. doi:10.4161/mabs.2.3.11779
15. Prabakaran P, Zhu Z, Chen W, Gong R, Feng Y, Streaker E, et al. Origin, diversity, and maturation of human antiviral antibodies analyzed by high-throughput sequencing. *Front Microbiol* (2012) **3**:277. doi:10.3389/fmicb.2012.00277
16. Mascola JR, Haynes BF. HIV-1 neutralizing antibodies: understanding nature's pathways. *Immunol Rev* (2013) **254**:225–44. doi:10.1111/imr.12075
17. Van Regenmortel MH, Andrieu JM, Dimitrov DS, Ensoli B, Hioe CE, Moog C, et al. Paradigm changes and the future of HIV vaccine research: a summary of a workshop held in Baltimore on 20 November 2013. *J AIDS Clin Res* (2014) **5**:1–5.
18. Zhu Z, Dimitrov DS. Construction of a large naive human phage-displayed Fab library through one-step cloning. *Methods Mol Biol* (2009) **525**:129–142, xv. doi:10.1007/978-1-59745-554-1_6
19. Prabakaran P, Chen W, Singarayan MG, Stewart CC, Streaker E, Feng Y, et al. Expressed antibody repertoires in human cord blood cells: 454 sequencing and IMGT/HighV-QUEST analysis of germline gene usage, junctional diversity, and somatic mutations. *Immunogenetics* (2012) **64**:337–50. doi:10.1007/s00251-011-0595-8
20. Alamyar E, Duroux P, Lefranc MP, Giudicelli V. IMGT((R)) tools for the nucleotide analysis of immunoglobulin (IG) and T cell receptor (TR) V-(D)-J repertoires, polymorphisms, and IG mutations: IMGT/V-QUEST and IMGT/HighV-QUEST for NGS. *Methods Mol Biol* (2012) **882**:569–604. doi:10.1007/978-1-61779-842-9_32
21. Hall TA. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucl Acids Symp Ser* (1999) **41**:95–8.
22. Han MV, Zmasek CM. phyloXML: XML for evolutionary biology and comparative genomics. *BMC Bioinformatics* (2009) **10**:356. doi:10.1186/1471-2105-10-356
23. Wu X, Zhou T, Zhu J, Zhang B, Georgiev I, Wang C, et al. Focused evolution of HIV-1 neutralizing antibodies revealed by structures and deep sequencing. *Science* (2011) **333**:1593–602. doi:10.1126/science.1207532
24. Scheid JF, Mouquet H, Ueberheide B, Diskin R, Klein F, Oliveira TY, et al. Sequence and structural convergence of broad and potent HIV antibodies that mimic CD4 binding. *Science* (2011) **333**:1633–7. doi:10.1126/science.1207227
25. Yu L, Guan Y. Immunologic basis for long HCDR3s in broadly neutralizing antibodies against HIV-1. *Front Immunol* (2014) **5**:250. doi:10.3389/fimmu.2014.00250
26. Hoot S, McGuire AT, Cohen KW, Strong RK, Hangartner L, Klein F, et al. Recombinant HIV envelope proteins fail to engage germline versions of anti-CD4bs bNAbs. *PLoS Pathog* (2013) **9**:e1003106. doi:10.1371/journal.ppat.1003106
27. McGuire AT, Hoot S, Dreyer AM, Lippy A, Stuart A, Cohen KW, et al. Engineering HIV envelope protein to activate germline B cell receptors of broadly neutralizing anti-CD4 binding site antibodies. *J Exp Med* (2013) **210**:655–63. doi:10.1084/jem.20122824
28. McGuire AT, Glenn JA, Lippy A, Stamatatos L. Diverse recombinant HIV-1 Envs fail to activate B cells expressing the germline B cell receptors of the broadly neutralizing anti-HIV-1 antibodies PG9 and 447-52D. *J Virol* (2014) **88**:2645–57. doi:10.1128/JVI.03228-13
29. Liao HX, Lynch R, Zhou T, Gao F, Alam SM, Boyd SD, et al. Co-evolution of a broadly neutralizing HIV-1 antibody and founder virus. *Nature* (2013) **496**:469–76. doi:10.1038/nature12053
30. Doria-Rose NA, Schramm CA, Gorman J, Moore PL, Bhiman JN, DeKosky BJ, et al. Developmental pathway for potent V1V2-directed HIV-neutralizing antibodies. *Nature* (2014) **509**:55–62. doi:10.1038/nature13036
31. Jardine J, Julien JP, Menis S, Ota T, Kalyuzhniy O, McGuire A, et al. Rational HIV immunogen design to target specific germline B cell receptors. *Science* (2013) **340**:711–6. doi:10.1126/science.1234150
32. Klein F, Diskin R, Scheid JF, Gaebler C, Mouquet H, Georgiev IS, et al. Somatic mutations of the immunoglobulin framework are generally required for broad and potent HIV-1 neutralization. *Cell* (2013) **153**:126–38. doi:10.1016/j.cell.2013.03.018
33. Zhou T, Zhu J, Wu X, Moquin S, Zhang B, Acharya P, et al. Multidonor analysis reveals structural elements, genetic determinants, and maturation pathway for HIV-1 neutralization by VRC01-class antibodies. *Immunity* (2013) **39**:245–58. doi:10.1016/j.immuni.2013.04.012
34. Zhu J, Ofek G, Yang Y, Zhang B, Louder MK, Lu G, et al. Mining the antibodyome for HIV-1-neutralizing antibodies with next-generation sequencing and phylogenetic pairing of heavy/light chains. *Proc Natl Acad Sci U S A* (2013) **110**:6470–5. doi:10.1073/pnas.1219320110
35. Zhu J, Wu X, Zhang B, McKee K, O'Dell S, Soto C, et al. De novo identification of VRC01 class HIV-1-neutralizing antibodies by next-generation sequencing of B-cell transcripts. *Proc Natl Acad Sci U S A* (2013) **110**:E4088–97. doi:10.1073/pnas.1306262110
36. Sok D, Laserson U, Laserson J, Liu Y, Vigneault F, Julien JP, et al. The effects of somatic hypermutation on neutralization and binding in the PGT121 family of broadly neutralizing HIV antibodies. *PLoS Pathog* (2013) **9**:e1003754. doi:10.1371/journal.ppat.1003754
37. Zhu Z, Qin HR, Chen W, Zhao Q, Shen X, Schutte R, et al. Cross-reactive HIV-1-neutralizing human monoclonal antibodies identified from a patient with 2F5-like antibodies. *J Virol* (2011) **85**:11401–8. doi:10.1128/JVI.05312-11
38. Ofek G, Zirkle B, Yang Y, Zhu Z, McKee K, Zhang B, et al. Structural basis for HIV-1 neutralization by 2F5-like antibodies m66 and m66.6. *J Virol* (2014) **88**:2426–41. doi:10.1128/JVI.02837-13
39. Goo L, Chohan V, Nduati R, Overbaugh J. Early development of broadly neutralizing antibodies in HIV-1-infected infants. *Nat Med* (2014) **20**:655–8. doi:10.1038/nm.3565
40. Glanville J, Zhai W, Berka J, Telman D, Huerta G, Mehta GR, et al. Precise determination of the diversity of a combinatorial antibody library gives insight

into the human immunoglobulin repertoire. *Proc Natl Acad Sci U S A* (2009) **106**:20216–21. doi:10.1073/pnas.0909775106
41. Furukawa K, Akasako-Furukawa A, Shirai H, Nakamura H, Azuma T. Junctional amino acids determine the maturation pathway of an antibody. *Immunity* (1999) **11**:329–38. doi:10.1016/S1074-7613(00)80108-9
42. West AP Jr, Diskin R, Nussenzweig MC, Bjorkman PJ. Structural basis for germ-line gene usage of a potent class of antibodies targeting the CD4-binding site of HIV-1 gp120. *Proc Natl Acad Sci U S A* (2012) **109**:E2083–90. doi:10.1073/pnas.1208984109
43. Ippolito GC, Schelonka RL, Zemlin M, Ivanov II, Kobayashi R, Zemlin C, et al. Forced usage of positively charged amino acids in immunoglobulin CDR-H3 impairs B cell development and antibody production. *J Exp Med* (2006) **203**:1567–78. doi:10.1084/jem.20052217
44. Schelonka RL, Zemlin M, Kobayashi R, Ippolito GC, Zhuang Y, Gartland GL, et al. Preferential use of DH reading frame 2 alters B cell development and antigen-specific antibody production. *J Immunol* (2008) **181**:8409–15. doi:10.4049/jimmunol.181.12.8409
45. Gu H, Kitamura D, Rajewsky K. B cell development regulated by gene rearrangement: arrest of maturation by membrane-bound D mu protein and selection of DH element reading frames. *Cell* (1991) **65**:47–54. doi:10.1016/0092-8674(91)90406-O
46. Benichou J, Glanville J, Prak ET, Azran R, Kuo TC, Pons J, et al. The restricted DH gene reading frame usage in the expressed human antibody repertoire is selected based upon its amino acid content. *J Immunol* (2013) **190**:5567–77. doi:10.4049/jimmunol.1201929
47. Zemlin M, Schelonka RL, Ippolito GC, Zemlin C, Zhuang Y, Gartland GL, et al. Regulation of repertoire development through genetic control of DH reading frame preference. *J Immunol* (2008) **181**:8416–24. doi:10.4049/jimmunol.181.12.8416
48. Georgiou G, Ippolito GC, Beausang J, Busse CE, Wardemann H, Quake SR. The promise and challenge of high-throughput sequencing of the antibody repertoire. *Nat Biotechnol* (2014) **32**:158–68. doi:10.1038/nbt.2782
49. Haynes BF, Kelsoe G, Harrison SC, Kepler TB. B-cell-lineage immunogen design in vaccine development with HIV-1 as a case study. *Nat Biotechnol* (2012) **30**:423–33. doi:10.1038/nbt.2197
50. Rathore U, Kesavardhana S, Mallajosyula VV, Varadarajan R. Immunogen design for HIV-1 and influenza. *Biochim Biophys Acta* (2014). doi:10.1016/j.bbapap.2014.05.010
51. Alam SM, Dennison SM, Aussedat B, Vohra Y, Park PK, Fernandez-Tejada A, et al. Recognition of synthetic glycopeptides by HIV-1 broadly neutralizing antibodies and their unmutated ancestors. *Proc Natl Acad Sci U S A* (2013) **110**:18214–9. doi:10.1073/pnas.1317855110

Conflict of Interest Statement: The Guest Associate Editor Marc H. V. Van Regenmortel declares that, despite having collaborated with the author Dimiter S. Dimitrov, the review process was handled objectively and no conflict of interest exists. The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Received: 14 July 2014; accepted: 05 August 2014; published online: 28 August 2014.
*Citation: Prabakaran P, Chen W and Dimitrov DS (2014) The antibody germline/maturation hypothesis, elicitation of broadly neutralizing antibodies against HIV-1 and cord blood IgM repertoires. Front. Immunol. **5**:398. doi: 10.3389/fimmu.2014.00398*
This article was submitted to HIV and AIDS, a section of the journal Frontiers in Immunology.

frontiers in
IMMUNOLOGY

REVIEW ARTICLE
published: 18 June 2014
doi: 10.3389/fimmu.2014.00289

Which antibody functions are important for an HIV vaccine?

Bin Su and Christiane Moog*

INSERM U1109, Fédération de Médecine Translationnelle de Strasbourg, Université de Strasbourg, Strasbourg, France

Edited by:
Marc H. V. Van Regenmortel, University of Strasbourg, France

Reviewed by:
Gabriella Scarlatti, Fondazione Centro San Raffaele, Italy
Kristina Broliden, Karolinska Institutet, Sweden
Marc H. V. Van Regenmortel, University of Strasbourg, France

***Correspondence:**
Christiane Moog, INSERM U1109, Fédération de Médecine Translationnelle de Strasbourg, Université de Strasbourg, 3 rue Koeberlé, Strasbourg 67000, France
e-mail: c.moog@unistra.fr

HIV antibody (Ab) functions capable of preventing mucosal cell-free or cell-to-cell HIV transmission are critical for the development of effective prophylactic and therapeutic vaccines. In addition to $CD4^+$ T cells, other potential HIV-target cell types including antigen-presenting cells (APCs) (dendritic cells, macrophages) residing at mucosal sites are infected. Moreover, the interactions between APCs and HIV lead to HIV cell-to-cell transmission. Recently discovered broadly neutralizing antibodies (NAbs) are able to neutralize a broad spectrum of HIV strains, inhibit cell-to-cell transfer, and efficiently protect from infection in the experimentally challenged macaque model. However, the 31% protection observed in the RV144 vaccine trial in the absence of detectable NAbs in blood samples pointed to the possible role of additional Ab inhibitory functions. Increasing evidence suggests that IgG Fcγ receptor (FcγR)-mediated inhibition of Abs present at the mucosal site may play a role in protection against HIV mucosal transmission. Moreover, mucosal IgA Abs may be determinant in protection against HIV sexual transmission. Therefore, defining Ab inhibitory functions that could lead to protection is critical for further HIV vaccine design. Here, we review different inhibitory properties of HIV-specific Abs and discuss their potential role in protection against HIV sexual transmission.

Keywords: HIV, mucosal HIV vaccine, cell-to-cell transfer, neutralizing antibodies, non-neutralizing inhibitory antibodies, FcγR, antigen-presenting cells, ADCC

INTRODUCTION

Sexual transmission is currently the major route of HIV infection worldwide. In more than 80% of newly diagnosed cases of HIV-1 infection, the patients become infected during sexual intercourse (1). This route of infection can be prevented by IgG neutralizing antibodies (NAbs) and secretory IgA (2, 3). Recently discovered potent and broadly NAbs (bNAbs) are able to neutralize a broad spectrum of cell-free and cell-associated HIV strains (4–13). These antibodies (Abs) have also been shown to efficiently protect non-human primates (NHP) and humanized mice from experimental challenge (14–20). However, bNAbs display very specific characteristics and are extremely difficult to induce since only 10–30% of patients develop such Abs (21–25) and attempts to induce them by vaccination have failed. bNAbs are characterized by uncommonly long complementarity-determining loops and extensive somatic hypermutation, suggesting the need for a long maturation process, which makes their induction by vaccination extremely difficult.

Interestingly, the limited 31% protection observed in the RV144 vaccine trial in the absence of detectable NAbs in plasma/serum specimens pointed to a possible role of additional Ab inhibitory functions in this protection (26, 27) and defining these additional functions is therefore critical. Increasing evidence suggests that IgG Fcγ receptor (FcγR)-mediated inhibition of Abs, leading to phagocytosis or antibody-dependent cellular cytotoxicity (ADCC), plays a role in protection. These FcγRs are expressed on various antigen-presenting cells (APCs) and natural killer (NK) cells present at the mucosal site, suggesting that Fc-mediated inhibitory functions may contribute to the blockage of mucosal transmission. These cells may play a decisive role during sexual transmission since they have been proposed to be the first HIV targets at the mucosal site (28–30). Evidence from *in vivo* studies showed that HIV-specific Abs displaying Fc-mediated inhibition in the absence of neutralizing activity is able to decrease the viral load after experimental vaginal challenge in the macaque model (31, 32). Besides, various Ab inhibitory functions at the mucosal site such as aggregation, complement inhibition, inhibition of HIV transfer, and inhibition by induction of antiviral cytokines and chemokines may also contribute to HIV protection. In addition to the induction of NAbs, new vaccination strategies based on such Ab activities, should be considered. In the present review, HIV inhibition by Abs based on these various potential inhibitory functions will be discussed, as well as its possible contribution to the development of new vaccination strategies.

HIV-1 TRANSMISSION THROUGH MUCOSAL TISSUES

Very little is known about how HIV infects and disseminates through mucosal tissues. The selection of transmitted/founder (T/F) virus occurs at the mucosal portal of HIV entry (33–38). Mucosal sites contain a variety of immune cells targeted by HIV, i.e., APCs comprising various types of dendritic cells (DCs), macrophages, NK cells, and CD4 T lymphocytes (28–30, 39–43) (**Figure 1**). However, the exact mechanism by which viral particles migrate through the epithelial barrier remains unclear. Various modes of infection have been proposed, which include transfer through epithelial cells and intestinal epithelium, transport of HIV *via* DCs present at mucosal surfaces, and direct infection of resident CD4 T cells (41, 44–48) (**Figure 1**). Apart from direct infection of immune cells by cell-free virus, cell-to-cell transmission has been suggested to play a major role in HIV propagation and dissemination *in vivo*. Spread of HIV infection by cell-to-cell

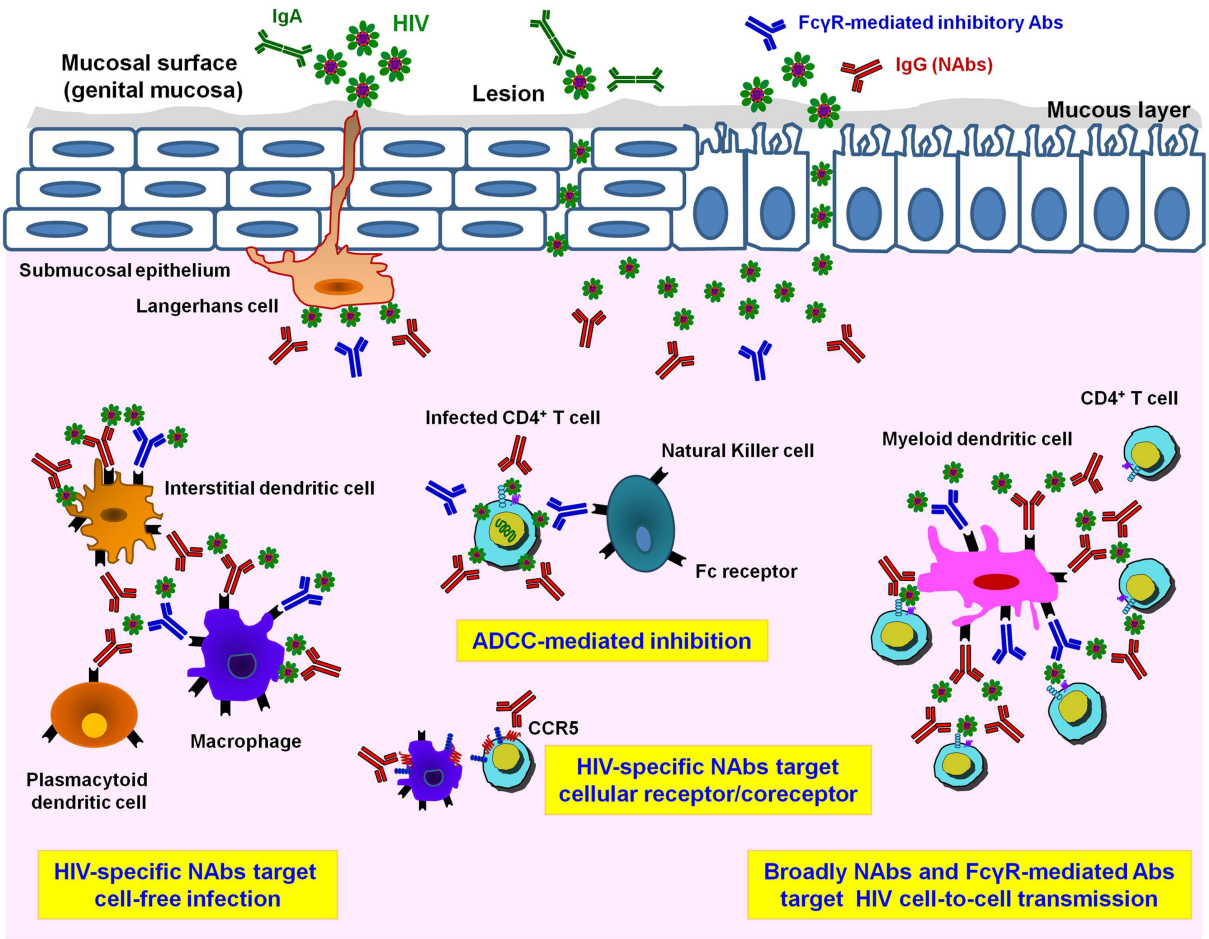


FIGURE 1 | Different HIV-1 antibody activities in mucosal tissues. Infectious HIV-1 particles can cross a multi-cellular layer of stratified squamous epithelial cells in genital mucosal tissues. Both cell-free and cell-associated HIV-1 virions infect host cells. Langerhans cells transport the virus into the sub-epithelium and mucosal lesions may provide an accessible pathway for HIV-1. In the sub-epithelium, in addition to target $CD4^+$ T cells, other potential HIV-target cell types including myeloid dendritic cells (DCs) and macrophages are infected either by cell-free virions or by cell-associated virions. Mucosal HIV-specific IgA (IgA, in green) can bind and neutralize cell-free virus at mucosal surfaces. Adaptive immune responses such as HIV-1-specific IgG neutralizing antibodies (NAbs, in red) are important for preventing HIV-1 cell-free infection. Only NAbs are able to inhibit HIV infection of CD4 T lymphocytes while both NAbs and FcγR-mediated inhibitory Abs (in blue) help to inhibit the spread of infection *via* cell-to-cell transmission route. Prevention of HIV-1 infection and killing of virus-producing cells by Ab-dependent mechanisms, especially antibody-dependent cellular cytotoxicity (ADCC) *via* binding of Fc receptors presented on the surface of innate immune cells such as natural killer (NK) cells, monocytes, DCs, or macrophages, takes place by inhibiting viral replication and diminishing viral reservoirs *in vivo*. Moreover, inhibitory NAbs directed to cellular target epitopes, such as CCR5 or other HIV-receptor/co-receptor structures, could provide additional targets for the rational design of novel vaccine candidates.

transmission has been found to be 100- to 1000-fold more efficient than infection by cell-free virions (49–54). At the mucosal level, in addition to CD4 T cells many cells are targeted by direct cell-free or cell-associated HIV-1 and the inhibition of these multiple routes of infection involve numerous immunological defenses (55), such as secretory IgA aggregation, Fc-mediated inhibition, neutralization of CD4 T cell infection, lysis of infected cells by NK cells, phagocytosis after antigen presentation, and inhibition following cytokine and chemokine production (**Figure 1**). For example, HIV-1 trapped by DCs can be inhibited by Fc-mediated inhibitory Abs, whereas inhibition of HIV-1 transfer from DCs to T cells will involve potent HIV NAbs (56). Therefore, in addition to neutralization of HIV-1-infected CD4 T cells by specific bNAbs, numerous additional inhibitory pathways, depending on the amount and type of HIV-1 and on the type of cells in the mucosa, may participate in HIV-1 inhibition and could decrease the concentration of NAbs necessary for protection.

MUCOSAL B-CELL RESPONSES

A major defensive mechanism from the mucosal immune system involves local production and secretion of IgG and dimeric or multimeric IgA from B-cells (57–60). The initial immune stimulation occurs mainly in mucosa-associated lymphoid tissues, particularly Peyer's patches of the distal ileum and other parts of gut-associated lymphoid tissues (61, 62). From these inductive sites the activated B-cells reach peripheral blood by migrating through lymph and draining lymph nodes and subsequently extravagate at secretory effector sites on a competitive basis depending on complementary

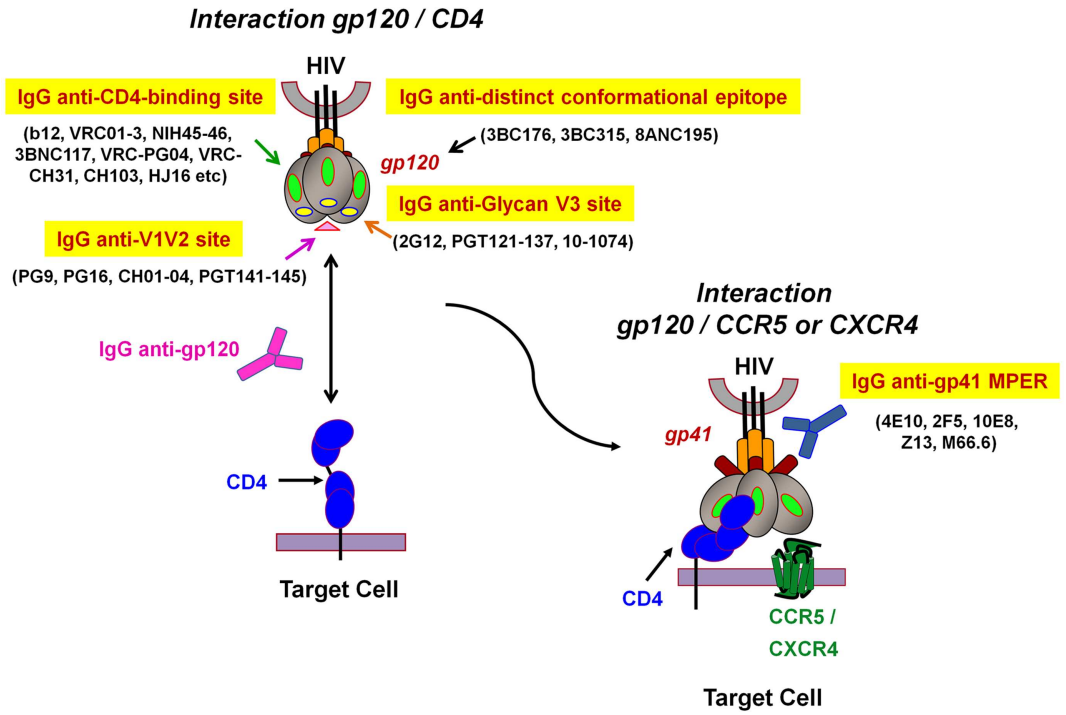


FIGURE 2 | Model representation of HIV-1 envelope glycoprotein structure and epitopes of broadly neutralizing antibodies. The surface receptor binding subunit gp120 and the fusion-mediating transmembrane subunit gp41 make up the functional HIV-1 envelope glycoproteins. The targets of broadly neutralizing antibodies (bNAbs) can be divided into several groups: (1) IgG anti-CD4-binding site, (2) IgG anti-V1V2 site, (3) IgG anti-*N*-linked glycan V3 site, and (4) IgG anti-gp41 membrane proximal external region (MPER). The IgG anti-distinct conformational epitope present on the envelope trimer, remains to be determined (adapted from Dr. Béatrice Labrosse).

adhesion molecules and chemokine–receptor pairs (61, 63). In addition, B-cells with "innate-like" functions including B-1 cells are enriched in mucosal tissues and marginal zone B-cells (64). These B-cells produce natural Abs that recognize conserved features of bacterial carbohydrates and phospholipids, that generate a first line of protection through the early production of low-affinity IgM in response to bacteria (62, 64–66). Mucosal DCs support B-cell activation and several factors in mucosal tissues, including both T cell-dependent and T cell-independent factors have been show to favor B-cell immunoglobulin class-switching to IgA-secreting plasma cells (59, 67). However, the exact local production sites and local redistribution at the mucosal site have not been well documented. During acute HIV infection phase, naïve B-cells are immediately decreased and reciprocal memory B-cell increased at mucosal sites and blood although little is known on the phenotypic features and functions of B-cell populations and early B-cell subversions occurring at mucosal sites (68). As most HIV-1 transmission occurs *via* mucosal sites, eliciting effective mucosal B-cell responses with long-lasting protective NAbs at mucosal sites is therefore critical to provide the first line of protection at mucosal surfaces for preventing early HIV-1 invasion by HIV-1 vaccine (69–71).

MECHANISMS OF INHIBITORY ACTIVITY OF NEUTRALIZING ANTIBODIES

Most HIV-1 vaccination strategies aim to induce human HIV-specific Abs able to inhibit the infection of target cells at the onset of viral transmission (2, 11, 72). Humoral responses against HIV have been extensively studied and NAbs able to efficiently neutralize *in vitro* a broad range of circulating HIV-1 strains have been described (10, 12, 20). These include the well-characterized NAb b12, 2G12, 447-52D, 2F5, 4E10, as well as novel bNAbs such as VRC01 and 10–1074 or belonging to PGT family that neutralize a large spectrum of HIV-1 isolates of various clades (4–7, 12, 73–75) (**Figure 2**). These Abs efficiently inhibit cell-free HIV primary isolates or pseudoviruses *in vitro* in conventional neutralization assays with peripheral blood mononuclear cells (PBMCs) or HIV-permissive cell lines (TZM-bl). Both assays assess the capacity of Abs to inhibit HIV-1 infection of either $CD4^+$ primary cells or TZM-bl cell lines that express the CD4 receptor and co-receptor CCR5. Abs possessing a neutralizing activity will recognize functionally important structures and conserved epitopes of the HIV viral envelope gp120 and gp41, and will impede virus attachment as well as fusion and entry processes that lead to a decrease in HIV replication (9–12) (**Figure 2**). The neutralization process is due to the capacity of Abs to directly inhibit HIV-1 replication in the absence of additional factors, such as Fc receptors (FcRs) or complement. Yet, due to the complex glycosylation profile of HIV and conformational changes of the viral envelope during fusion (**Figure 2**), most NAbs require long HCDR3s to allow the recognition of poorly accessible conserved Env epitopes (76). Moreover, NAbs isolated from infected patients result from a long maturation and somatic hypermutation processes (9–12). These unusual Ab characteristics will unfortunately be extremely difficult

to generate by vaccination. Several of these HIV-1 bNAbs have been reverted experimentally to their unmutated ancestral state, and were found to bind weakly or undetectably to native HIV-1 Env (77, 78), which means that Ab responses induced by vaccination will have to occur following intricate pathways of B-cell maturation.

Recent studies showed that high levels of IgG Abs specific for the first and second variable regions (V1V2) of gp120 were inversely associated with a reduced risk of HIV-1 infection in the RV144 clinical vaccine trial (27, 79–81). Moreover, Yates et al. recently found that vaccine-induced HIV-1-specific IgG3 responses correlated with decreased risk of infection in RV144 clinical trial compared to the VAX003 vaccine regimen (82). Since partial protection observed in the RV144 phase III Thailand trial was mediated by the induction of non-neutralizing antibodies (NNAbs) and a moderate T cell response (27, 83), it seems that other immune mechanisms in addition to classical NAbs responses are required to achieve protection against HIV infection.

MECHANISMS OF Fc RECEPTOR-MEDIATED PROTECTION

The FcR-dependent mechanism of inhibition has been observed in various HIV-target cells that express these receptors, for instance DCs, Langerhans cells, and macrophages (56, 84–90). HIV inhibition involving interactions with FcR receptors was confirmed with the cell line TZM-bl that expresses various FcγRs (91, 92) mainly FcγRI and FcγRII (84, 85, 91). Fc-mediated inhibition increased by 10- to 1000-fold the inhibitory activity of NAbs in FcR-bearing macrophages (84), and neutralization titers of NAbs 4E10 and 2F5 were increased as much as 5000-fold in the case of TZM-bl cells expressing FcγRI (91, 92). Some HIV-1-specific Abs lacking neutralizing activities have also been shown to display Fc-mediated inhibitory activities (93). Such Abs, which inhibit HIV-1 replication only *via* FcγR receptors are referred to as non-neutralizing inhibitory Abs (NNIAbs) (85). In the case of APCs bearing FcγR, the formation of immune complexes between Abs and HIV leads to phagocytosis of the virus and its degradation by specific lysosomes (88, 94–96). Moreover, the fixation of Abs on the FcR of effector cells can also induce antiviral cytokines and chemokines, further impeding viral replication (97, 98). The mechanism of inhibition of NNIAbs implies that, contrary to NAbs, they do not need to recognize functional Env spikes. NNIAbs capture the virus *via* the Fab domains and bind to FcR-bearing cells *via* their Fc domains, increasing therefore the number of potential epitopes susceptible to be targeted by immunogens.

Recently, it has been shown that V1V2-specific IgG3 subclass Abs are associated with broad antiviral responses and were correlated with a decreased risk of infection in the RV144 vaccine trial (82). Chung et al. also found that in this trial, NNAbs were induced that presented highly coordinated Fc-mediated effector responses by the selective induction of highly functional IgG3 (99). These studies indicate that functional activity and Ab subclass may contribute to the potential antiviral activity of Abs that extends beyond virus neutralization and illustrate the potential role of FcγR-mediated innate and adaptive immune functions in additional HIV-1 protective mechanisms.

ANTIBODY-DEPENDENT CELLULAR CYTOTOXICITY

Antibody-dependent cellular cytotoxicity was reported in HIV-infected patients in 1987, when it was shown that HIV envelope gp120 bound to CD4 T cells was sensitive to lysis by PBMCs from HIV-infected patients (100). This ADCC mechanism also involves FcRs (93, 101), mainly FcRIII (CD16). Cross linking of Abs that recognize an infected target cell *via* its Fab domain and the FcR on the effector cell *via* its Fc domain leads to lysis of the infected target cell subsequent to effector cell degranulation (101–105). Various immune cells such as NK cells, monocytes, macrophages, or neutrophils can induce ADCC (106). It has been suggested that ADCC participated in the 31% reduced risk of HIV infection in the RV144 trial (26). Recent studies showed that ADCC also occurred in elite controllers (107, 108). Even though a correlation between *in vitro* ADCC and protection was not demonstrated, there is evidence that ADCC might account, at least partially, for protection against SHIV/SIV challenge in the *in vivo* macaque model (109). Hence, inducing Abs with ADCC function might enhance protection and should be considered as a goal in future vaccine approaches.

ANTIBODY-DEPENDENT CELL-MEDIATED VIRUS INHIBITION ACTIVITY

Antibody-dependent cell-mediated virus inhibition (ADCVI) results from an interaction between an infected target cell and an effector cell expressing one or several FcγRs *via* an HIV-specific Ab. ADCVI encompasses multiple effector functions related to lytic (e.g., ADCC) and non-cytolytic (e.g., production of β-chemokines) mechanisms leading to a decreased HIV-1 infection and replication (31, 93, 95, 110).

ROLE OF IgA-MEDIATED INHIBITION

In patients infected with HIV-1, a specific IgA response develops in parallel to the IgG response. Noteworthy, anti-gp41 (but not anti-gp120) IgA Abs were frequently elicited in both plasma and mucosal fluids within the first weeks after transmission. However, shortly after induction, these initial mucosal anti-gp41 Env IgA Abs rapidly declined (111). Later on, during the chronic phase, virus-specific IgA are low in both mucosa and systemic compartments (112). Interestingly, HIV-specific IgA are detected in the genital tract or the seminal fluid in seronegative (in the absence of serological HIV-specific IgG) partners of HIV-positive subjects "highly exposed persistently seronegative" (HEPS) (113–115). Mazzoli et al. first showed that IgA was detected in urine and vaginal samples from HIV-exposed seronegative individuals in the absence of IgG detection (113). In addition, virus-specific IgAs were detected in the salivary secretions of children from seropositive mothers. The presence of IgA in seronegative subjects that are in regular contact with HIV suggests its potential role in protection.

In vitro, IgA recapitulates for some Abs the neutralizing activity of IgG. In the case of the epitope recognized by NAb b12, the neutralizing activity of IgA was equivalent to that of the IgG (116). Furthermore, IgA displayed an additional inhibitory function involving its Fc region distinct to that of IgG. The presence of IgA at mucosal sites may involve a local activation of different immune mechanisms, such as a secretory component of IgA-mediated protection of mucus-bound IgA *in vivo* (117, 118), the aggregation of

secretory IgA (119), or mechanisms that involve ADCC effectors cells expressing FcαR (such as neutrophils). Recent results from the RV144 vaccine trial demonstrated that the levels of vaccine-induced IgA in serum were associated with a lack of protection against HIV acquisition (27) and that IgA competed with IgG for ADCC activity (120). Anti-HIV IgA therefore interferes with a protective IgG function, impeding its protective potential. On contrary, it was recently shown that the anti-HIV IgA1 isotype protected macaques better than the corresponding IgA2 or IgG Ab types (3). These findings suggest that, depending on their localization and/or structure, vaccine-induced or pre-existing IgA may have either a deleterious effect by competing with potential IgG protective Abs or a significant protective effect by limiting HIV transmission at the mucosal site (3). This dual IgA activity illustrates the complexity of Ab functions, that depend on the cellular and cytokine environment.

MECHANISMS OF INHIBITION OF HIV CELL-TO-CELL TRANSMISSION

Numerous studies suggest that direct cell-to-cell transmission occurring early at the mucosal site after sexual transmission makes a major contribution to rapid HIV-1 dissemination throughout the body. This mode of transmission has important consequences for designing treatments or vaccine strategies as inhibition of this type of HIV spread is even more complex than cell-free infection. Inhibitory activity of cell-to-cell spread may depend on donor and target cells such as APC-to-T cell or T cell-to-T cell, on viral strains, multiplicity of viral infection, etc. Consequently, results may diverge and may be controversial (121, 122).

Efficient HIV transmission occurs mainly *via* the formation of virological synapses (123–126). APC-to-T cell and T cell-to-T cell transfer experiments have been used to analyze the inhibitory activity of specific anti-HIV Abs in HIV transmission. By dissecting the early steps of HIV-1 spread from DCs to autologous primary CD4 T lymphocytes, it was shown that NAbs were able to efficiently inhibit HIV-1 transmission to CD4 T lymphocytes (56). Similar inhibitory activities by Abs have also been observed by others (127–131), suggesting that HIV-1 transfer from DCs to T lymphocytes can be affected by Ab inhibition. Furthermore, Fc-mediated inhibitory activity of Abs on the infection of APCs may decrease HIV-1 transmission to surrounding T cells. This is particularly relevant for DCs and macrophages as these cells highly express FcRs (56) [reviewed in Ref. (122)]. Abs can bind FcRs and therefore inhibit HIV-1 transmission *via* FcR-mediated inhibitory activity. Some NNIAbs such as 246-D have been found to reduce significantly the percentage of infected DCs *in vitro* (56). For these Abs, a strong association was found between FcγR-specific binding capacity, inhibition of HIV-1 replication, and DC maturation. This indicates that the binding of these Abs to DCs induces the maturation of these cells, resulting in lower levels of R5 virus replication (56). However, other authors observed a drastic decrease of Ab inhibitory activity in HIV transfer conditions (51, 54, 132–134). Of note, these later studies mainly involved cell lines in *in vitro* transfer protocols and the characteristics of the cell-to-cell contact appeared to be determinant for HIV inhibition (54). Close interactions between donor/target cells and immunological synapse formation differ according to the type of cells. DC/lymphocyte crosstalk involves ICAM-1 and LFA-1 adhesion molecules and stabilize interactions (135–139) that are absent with TZM-bl cell lines (140, 141). As a result, the strength of the established synapse will influence the efficiency of HIV spread, and the subsequently inhibitory potential of Abs (54, 56). APC/lymphocyte crosstalk can also modulate the immune response (126, 139, 142–146). Close contact between cells in particular tissues need therefore to be taken into consideration when analyzing HIV transfer. However, very little is currently known about the efficacy of HIV spread and the potency of HIV-specific Abs in different tissue environments.

In addition, during chronic infection, HIV replication propagates in lymphoid organs containing numerous $CD4^+$ T lymphocytes (147, 148) and cell-to-cell transmission between T cells is likely to be the most common mode of HIV-1 spread (124, 146, 149–152). HIV inhibitory activity of Abs on T cell-to-T cell transmission has been extensively studied (51, 53, 54, 133, 153–158), and variable inhibitory activities have been recorded depending on the type of T cells and virus used. These discrepancies emphasize the necessity to further investigate functional Ab activities in the context of HIV spread in tissues and lymphoid organs.

ENHANCING ANTIBODIES

Enhancing Abs were first described as complement-mediated enhancement by Robinson et al. (159, 160). Such Abs, unlike neutralizing or inhibitory Abs, facilitate infection by HIV *in vitro* by increasing HIV titers (e.g., an increase in the number of infected cells) or by augmenting the production of infectious virus particles. Ab-dependent enhancement of HIV-1 binding and infection of certain cell types has been demonstrated in different *in vitro* protocols (160–163). However, the mechanism leading to this increase has not been clearly identified although it has been proposed that Fc–Fcγ receptor interactions or conformational changes in Env or complement receptors may play a role (164–166). The HIV-1/IgG complex is able to bind to FcRs and it could therefore be transcytosed by APCs (167). It has also been proposed that virus coated with Abs and taken up *via* the FcR on DCs may lead to enhancement by FcR-mediated transcytosis of the virus–IgG complex (163). Recently, it was shown that the binding of HIV-1-specific Abs to neonatal FcR expressed on epithelial cells could enhance transcytosis of HIV-1 at low pH (168). Since neonatal FcR was detected in areas of the genital tract that are potentially exposed to HIV-1 during sexual intercourse, this new model of Ab-dependent enhancement points to an additional mechanism by which sexual transmission of HIV-1 may be facilitated (168). However, no facilitating role of Abs has yet been demonstrated *in vivo* for HIV infection following vaccination or HIV disease progression (169). There was no evidence of an increased HIV infection among vaccine recipients in the VaxGen and RV144 phase III vaccine trials (26, 170). It should be noted that a recent study showed a correlation between the presence of a particular allele of FcγR and an increased risk of infection in a sub-group of volunteers with low risk practices (171). These results suggest a possible deleterious effect of specific HIV Abs in a subpopulation of patients with a particular FcγR genotype. Therefore, supplemental studies need to be conducted in future prophylactic vaccine trials in order to circumvent any possible deleterious enhancing effects, since vaccination obviously should avoid the induction of such Abs.

MECHANISMS OF INHIBITION OF HIV SPREAD *IN VIVO* BY ANTIBODIES

The protective role of HIV-specific Abs has been extensively studied in various experimental models of infection, including NHP models (17, 19, 31, 172–178), and humanized mice models (14–16). The potential role of FcγR-mediated innate and adaptive immune functions in addition to neutralization has been repeatedly demonstrated in HIV protection (32, 95, 101, 110, 174, 179). Neutralizing monoclonal IgG1 b12, devoid of Fc–FcγR functions has decreased protective potential following vaginal challenge in NHPs (110). NNIAbs were able to reduce the viral load in challenged macaques without conferring complete protection (31, 32). These observations clearly indicate that, in addition to neutralization, FcγRs are important for achieving protection *in vivo*. Such effective protection observed *in vivo* suggests that HIV-specific Abs inhibit infection by cell-free virus and cell-to-cell transmission (**Figure 1**), both mechanisms contributing to HIV-1 replication and dissemination in the body. Interestingly, the Ab threshold necessary for sterilizing protection decreased in the animal model with decreased virus challenge (174). This further suggests that Abs may display increased potential during sexual transmission in the mucosal environment in the presence of low virus input. In this case, the balance in favor of HIV protection may be more easily achieved by vaccination, as suggested by the partial protection in the RV144 trial observed in a low risk population.

NOVEL UNRAVELED MECHANISM OF ANTIBODY INHIBITION

Recently, another Ab inhibitory activity was reported that provides protection inside cells by triggering an intracellular immune response in addition to extracellular activities (180). This activity was named ADIN for antibody-dependent intracellular neutralization (181). Working with a non-enveloped virus-like adenovirus as model, it was shown that Abs that bind virus before infection were carried into the cell while attached to the virus particle. Upon escape from the endosomal compartment, these Abs remain bound to the virus allowing it to be detected by the cell. Ab-coated virions are detected by a cytosolic intracellular Ab receptor called TRIM21, which binds to IgG with a higher affinity than any other Ab receptor so far described in humans (180, 182, 183). In addition to its Ab-binding domain, TRIM21 possesses a RING domain with E3 ubiquitin ligase activity. Using this ubiquitination activity, TRIM21 flags the virion for destruction by a mechanism involving proteasomal degradation. This process is very rapid and leads to removal of the virus before transcription and translation of the viral genome, in effect clearing the cell of infection (181). Moreover, it has been shown recently that the TRIM21-mediated ability of antisera to block replication was a consistent feature of the humoral immune response in immunized mice. In the presence of immune sera and upon infection, TRIM21 also activates a proinflammatory response, resulting in secretion of tumor necrosis factor alpha (TNF-α) and interleukin-6 (IL-6) (184). These results demonstrate that TRIM21 provides a potent block to the spreading of infection and induces an antiviral state (184). However, such Ab inhibitory activity may not be relevant to HIV since HIV is an enveloped virus that is uncoated following its entry in host cells. However, if Abs against the core proteins are endocytosed during infection, they may impair later intracellular HIV replication steps. Such an intracellular mechanism may explain some unexpected association between high anti-p24 Ab concentration and decreased viral load (185, 186).

Even more intriguing, broader Ab activities have recently been proposed. The group of Nancy Haigwoog showed an increase of the specific B-cell response, following the passive transfer HIV Abs in a NHP model (187). Using the FrCas mouse retroviral model, Michaud et al. observed a protection linked to the induction of long-term B and T response, due to passive transfer of NAbs (188). This mechanism of stimulation of the adaptive response following Abs transfer was also observed following NAb therapy in infected macaques (17). Such prolonged protection by induction of adaptive immune response by Abs was already described in cancer field (189). These studies attribute an "immunogenic" role to the Abs in that they would be able to induce primary and memory responses more efficiently than free viral particles or infected cells. In this way, Abs could participate in the implementation of an adaptive response, paving the way to new fields of applications.

PROMISE OF HIV ANTIBODIES IN AIDS VACCINES

Currently, one of the innovating vaccination strategies would consist in developing a mucosal vaccine as an effective means of prevention against HIV sexual transmission (72). The newly identified potent bNAbs that suppress active infections and clear infected cells in humanized mice and macaques suggest that these bNAbs would effectively protect from infection (20). However, in the development of a vaccine against HIV, the possibility of inducing such NAbs have been compromised when it was discovered that they possess unusual characteristics (heavy long chain HCDR3, significant numbers of somatic mutations) that require a long maturation and makes them difficult to induce. The maturation of progenitor B-cells is unlikely to be reproduced by a short stimulation with a single immunogen (8). Vaccination strategies based on a succession of immunogens that would be able to mimic, step by step, the process of maturation, and activation of B-cell clones are currently being tested. However, this approach of vaccination has never been implemented previously and in view of the complex mechanisms that are involved, it is unclear whether it will be successful.

In view of the major, constraints linked to the *in vivo* induction of NAbs, vaccine approaches involving the optimization of inhibitory Abs, induction of additional immune mechanisms, are currently being examined. These are similar to approaches followed in cancer research, and attempt to modify the Fc region of Abs in order to increase their inhibitory activity. Moldt et al. generated by mutagenesis and by modifying the glycosylation of the Fc region, a panel of mutants of the NAb b12, which retained the neutralizing activity of the Fab region but had different affinity the FcγRs (190, 191). These Fc modifications increased the affinity of Abs for FcγRs as well as the associated *in vivo* inhibitory functions (phagocytosis, ADCC, etc.). However, no improvement of protection was observed in experimentally challenged macaques.

By inducing inhibitory Abs directly at the sites of infection (anal mucosa, genital tracts, etc.), it might be possible to limit viral replication earlier and in many target cells. New immunogens are currently being formulated in order to redirect the humoral response to mucosal sites (192). A phase 1 clinical trial has recently

started (European collaborative project EuroNeut41) in order to test this new concept (193). However, these protocols are based on information gathered from the mouse model and local mucosal immune activation could not be reproduced in humans. Unfortunately, our current knowledge on how we could bring the immune response to converge toward mucosa is extremely limited and the mechanisms of action of anti-HIV Abs at mucosal sites are also poorly understood.

In addition to their central role in vaccination, Abs are also being investigated as possible therapeutic agents. Recent studies demonstrate that combinations of cocktails of two or more monoclonal Abs significantly reduced viremia in chronically infected macaques, suggesting that such therapies might be effective in humans (17, 19, 32). By combining diverse Abs properties to potentiate the protective effects of anti-HIV-specific Ab-based strategies, it might be possible to enhance what was achieved with antiviral compounds by inducing complementary inhibitory potentials gathered by Abs inhibitory functions. A combination of conventional multi-hits antiretroviral therapy with NAbs therapy might be successful and could generate revolutionary drug combinations that may lead to an HIV cure.

SUMMARY AND CONCLUSION

The last decade has witnessed enormous advances in our knowledge of HIV vaccine designs and trials. Although a large number of broadly and potent NAbs have been recently discovered (**Figure 2**), inducing such bNAbs by vaccination is likely to be very difficult (5, 7, 10). Data from *in vivo* studies and recent findings following clinical assays have demonstrated the importance of Fc-mediated Ab-dependant mechanisms in achieving protection against HIV. Therefore, new vaccination strategies including the induction of such type of activities, in addition to NAbs, should be developed. As HIV transmission at mucosal sites involves specific HIV targets, vaccination should induce an immune response that protects all the different potential mucosal target cells (i.e., using Abs that display different inhibitory activities). Moreover, vaccination should induce Abs and B-cell responses directly at mucosal level in order to rapidly interfere with the early events of HIV infection. Almost nothing is known about the local immune induction at mucosal sites, known to be involved in induction of tolerance. Strategies to develop local immune responses should therefore be encouraged as well as specific adjuvants and immunogens active at the mucosal site leading to a strong and long-lasting response. Furthermore, the protective role of HIV-specific Abs against cell-to-cell transmission should be evaluated by analyzing the transfer of transmitted/founder HIV. It is hoped that improved understanding of HIV transmission *via* cell-free or cell-associated models and of different functionalities of HIV-specific Abs may lead to a new generation of immunogens and immunotherapeutics for the development of protective and safe vaccine approaches.

ACKNOWLEDGMENTS

We would like to thank all our colleagues from the INSERM Unit 1110, especially Dr. Maryse Peressin for fruitful discussions; Sylvie Schmidt, Géraldine Laumond, and Thomas Decoville for excellent technical assistance. **Figure 2** was adapted from Dr. Béatrice Labrosse (INSERM Unit 941), thanks for this generous gift and her support. We thank our financial support from EuroNeut41 (FP7-HELTH-2007-A-201038), EuroPrise (LSHP-CT-2006-037611), VRI (Vaccine Research Institute), Sidaction, ANRS, Dormeur Investment Service, Ltd., and Fonds de Dotation Pierre Berge. Bin Su was supported by a fellowship from ANRS. We apologize to the authors and colleagues whose work is not cited because of space limitations.

REFERENCES

1. UNAIDS. *Global Report: UNAIDS Report on the Global AIDS Epidemic 2012.* Geneva: WHO (2012).
2. Mascola JR, Montefiori DC. The role of antibodies in HIV vaccines. *Annu Rev Immunol* (2010) **28**:413–44. doi:10.1146/annurev-immunol-030409-101256
3. Watkins JD, Sholukh AM, Mukhtar MM, Siddappa NB, Lakhashe SK, Kim M, et al. Anti-HIV IgA isotypes: differential virion capture and inhibition of transcytosis are linked to prevention of mucosal R5 SHIV transmission. *AIDS* (2013) **27**:F13–20. doi:10.1097/QAD.0b013e328360eac6
4. Walker LM, Phogat SK, Chan-Hui PY, Wagner D, Phung P, Goss JL, et al. Broad and potent neutralizing antibodies from an African donor reveal a new HIV-1 vaccine target. *Science* (2009) **326**:285–9. doi:10.1126/science.1178746
5. Wu X, Yang ZY, Li Y, Hogerkorp CM, Schief WR, Seaman MS, et al. Rational design of envelope identifies broadly neutralizing human monoclonal antibodies to HIV-1. *Science* (2010) **329**:856–61. doi:10.1126/science.1187659
6. Scheid JF, Mouquet H, Ueberheide B, Diskin R, Klein F, Oliveira TY, et al. Sequence and structural convergence of broad and potent HIV antibodies that mimic CD4 binding. *Science* (2011) **333**:1633–7. doi:10.1126/science.1207227
7. Walker LM, Huber M, Doores KJ, Falkowska E, Pejchal R, Julien JP, et al. Broad neutralization coverage of HIV by multiple highly potent antibodies. *Nature* (2011) **477**:466–U117. doi:10.1038/nature10373
8. Haynes BF, Kelsoe G, Harrison SC, Kepler TB. B-cell-lineage immunogen design in vaccine development with HIV-1 as a case study. *Nat Biotechnol* (2012) **30**:423–33. doi:10.1038/nbt.2197
9. Kwong PD, Mascola JR. Human antibodies that neutralize HIV-1: identification, structures, and B cell ontogenies. *Immunity* (2012) **37**:412–25. doi:10.1016/j.immuni.2012.08.012
10. Klein F, Mouquet H, Dosenovic P, Scheid JF, Scharf L, Nussenzweig MC. Antibodies in HIV-1 vaccine development and therapy. *Science* (2013) **341**:1199–204. doi:10.1126/science.1241144
11. Kwong PD, Mascola JR, Nabel GJ. Broadly neutralizing antibodies and the search for an HIV-1 vaccine: the end of the beginning. *Nat Rev Immunol* (2013) **13**:693–701. doi:10.1038/nri3516
12. Mascola JR, Haynes BF. HIV-1 neutralizing antibodies: understanding nature's pathways. *Immunol Rev* (2013) **254**:225–44. doi:10.1111/imr.12075
13. Gruell H, Klein F. Opening fronts in HIV vaccine development: tracking the development of broadly neutralizing antibodies. *Nat Med* (2014) **20**:478–9. doi:10.1038/nm.3567
14. Klein F, Gaebler C, Mouquet H, Sather DN, Lehmann C, Scheid JF, et al. Broad neutralization by a combination of antibodies recognizing the CD4 binding site and a new conformational epitope on the HIV-1 envelope protein. *J Exp Med* (2012) **209**:1469–79. doi:10.1084/jem.20120423
15. Klein F, Halper-Stromberg A, Horwitz JA, Gruell H, Scheid JF, Bournazos S, et al. HIV therapy by a combination of broadly neutralizing antibodies in humanized mice. *Nature* (2012) **492**:118–22. doi:10.1038/nature11604
16. Veselinovic M, Neff CP, Mulder LR, Akkina R. Topical gel formulation of broadly neutralizing anti-HIV-1 monoclonal antibody VRC01 confers protection against HIV-1 vaginal challenge in a humanized mouse model. *Virology* (2012) **432**:505–10. doi:10.1016/j.virol.2012.06.025
17. Barouch DH, Whitney JB, Moldt B, Klein F, Oliveira TY, Liu J, et al. Therapeutic efficacy of potent neutralizing HIV-1-specific monoclonal antibodies in SHIV-infected rhesus monkeys. *Nature* (2013) **503**:224–8. doi:10.1038/nature12744
18. McCoy LE, Weiss RA. Neutralizing antibodies to HIV-1 induced by immunization. *J Exp Med* (2013) **210**:209–23. doi:10.1084/jem.20121827
19. Shingai M, Nishimura Y, Klein F, Mouquet H, Donau OK, Plishka R, et al. Antibody-mediated immunotherapy of macaques chronically infected with SHIV suppresses viraemia. *Nature* (2013) **503**:277–80. doi:10.1038/nature12746

20. West AP Jr, Scharf L, Scheid JF, Klein F, Bjorkman PJ, Nussenzweig MC. Structural insights on the role of antibodies in HIV-1 vaccine and therapy. *Cell* (2014) **156**:633–48. doi:10.1016/j.cell.2014.01.052
21. Moog C, Fleury HJ, Pellegrin I, Kirn A, Aubertin AM. Autologous and heterologous neutralizing antibody responses following initial seroconversion in human immunodeficiency virus type 1-infected individuals. *J Virol* (1997) **71**:3734–41.
22. Burrer R, Salmon-Ceron D, Richert S, Pancino G, Spiridon G, Haessig S, et al. Immunoglobulin G (IgG) and IgA, but also nonantibody factors, account for in vitro neutralization of human immunodeficiency virus (HIV) type 1 primary isolates by serum and plasma of HIV-infected patients. *J Virol* (2001) **75**:5421–4. doi:10.1128/JVI.75.11.5421-5424.2001
23. Binley JM, Lybarger EA, Crooks ET, Seaman MS, Gray E, Davis KL, et al. Profiling the specificity of neutralizing antibodies in a large panel of plasmas from patients chronically infected with human immunodeficiency virus type 1 subtypes B and C. *J Virol* (2008) **82**:11651–68. doi:10.1128/JVI.01762-08
24. Sather DN, Armann J, Ching LK, Mavrantoni A, Sellhorn G, Caldwell Z, et al. Factors associated with the development of cross-reactive neutralizing antibodies during human immunodeficiency virus type 1 infection. *J Virol* (2009) **83**:757–69. doi:10.1128/JVI.02036-08
25. Simek MD, Rida W, Priddy FH, Pung P, Carrow E, Laufer DS, et al. Human immunodeficiency virus type 1 elite neutralizers: individuals with broad and potent neutralizing activity identified by using a high-throughput neutralization assay together with an analytical selection algorithm. *J Virol* (2009) **83**:7337–48. doi:10.1128/JVI.00110-09
26. Rerks-Ngarm S, Pitisuttithum P, Nitayaphan S, Kaewkungwal J, Chiu J, Paris R, et al. Vaccination with ALVAC and AIDSVAX to prevent HIV-1 infection in Thailand. *N Engl J Med* (2009) **361**:2209–20. doi:10.1056/NEJMoa0908492
27. Haynes BF, Gilbert PB, McElrath MJ, Zolla-Pazner S, Tomaras GD, Alam SM, et al. Immune-correlates analysis of an HIV-1 vaccine efficacy trial. *N Engl J Med* (2012) **366**:1275–86. doi:10.1056/NEJMoa1113425
28. Shattock RJ, Moore JP. Inhibiting sexual transmission of HIV-1 infection. *Nat Rev Microbiol* (2003) **1**:25–34. doi:10.1038/nrmicro729
29. Hladik F, Sakchalathorn P, Ballweber L, Lentz G, Fialkow M, Eschenbach D, et al. Initial events in establishing vaginal entry and infection by human immunodeficiency virus type-1. *Immunity* (2007) **26**:257–70. doi:10.1016/j.immuni.2007.01.007
30. Piguet V, Steinman RM. The interaction of HIV with dendritic cells: outcomes and pathways. *Trends Immunol* (2007) **28**:503–10. doi:10.1016/j.it.2007.07.010
31. Burton DR, Hessell AJ, Keele BF, Klasse PJ, Ketas TA, Moldt B, et al. Limited or no protection by weakly or nonneutralizing antibodies against vaginal SHIV challenge of macaques compared with a strongly neutralizing antibody. *Proc Natl Acad Sci U S A* (2011) **108**:11181–6. doi:10.1073/pnas.1103012108
32. Moog C, Dereuddre-Bosquet N, Teillaud JL, Biedma ME, Holl V, Van Ham G, et al. Protective effect of vaginal application of neutralizing and nonneutralizing inhibitory antibodies against vaginal SHIV challenge in macaques. *Mucosal Immunol* (2014) **7**:46–56. doi:10.1038/mi.2013.23
33. Keele BF, Giorgi EE, Salazar-Gonzalez JF, Decker JM, Pham KT, Salazar MG, et al. Identification and characterization of transmitted and early founder virus envelopes in primary HIV-1 infection. *Proc Natl Acad Sci U S A* (2008) **105**:7552–7. doi:10.1073/pnas.0802203105
34. Wilen CB, Parrish NF, Pfaff JM, Decker JM, Henning EA, Haim H, et al. Phenotypic and immunologic comparison of clade B transmitted/founder and chronic HIV-1 envelope glycoproteins. *J Virol* (2011) **85**:8514–27. doi:10.1128/JVI.00736-11
35. Ochsenbauer C, Edmonds TG, Ding H, Keele BF, Decker J, Salazar MG, et al. Generation of transmitted/founder HIV-1 infectious molecular clones and characterization of their replication capacity in CD4 T lymphocytes and monocyte-derived macrophages. *J Virol* (2012) **86**:2715–28. doi:10.1128/JVI.06157-11
36. Harman AN, Kim M, Nasr N, Sandgren KJ, Cameron PU. Tissue dendritic cells as portals for HIV entry. *Rev Med Virol* (2013) **23**:319–33. doi:10.1002/rmv.1753
37. Parker ZF, Iyer SS, Wilen CB, Parrish NF, Chikere KC, Lee FH, et al. Transmitted/founder and chronic HIV-1 envelope proteins are distinguished by differential utilization of CCR5. *J Virol* (2013) **87**:2401–11. doi:10.1128/JVI.02964-12
38. Parrish NF, Gao F, Li H, Giorgi EE, Barbian HJ, Parrish EH, et al. Phenotypic properties of transmitted founder HIV-1. *Proc Natl Acad Sci U S A* (2013) **110**:6626–33. doi:10.1073/pnas.1304288110
39. Haase AT. Targeting early infection to prevent HIV-1 mucosal transmission. *Nature* (2010) **464**:217–23. doi:10.1038/nature08757
40. Mesman AW, Geijtenbeek TB. Pattern recognition receptors in HIV transmission. *Front Immunol* (2012) **3**:59. doi:10.3389/fimmu.2012.00059
41. Cunningham AL, Harman A, Nasr N. Initial HIV mucosal infection and dendritic cells. *EMBO Mol Med* (2013) **5**:658–60. doi:10.1002/emmm.201202763
42. Xu H, Wang X, Veazey RS. Mucosal immunology of HIV infection. *Immunol Rev* (2013) **254**:10–33. doi:10.1111/imr.12072
43. Cavarelli M, Scarlatti G. HIV-1 infection: the role of the gastrointestinal tract. *Am J Reprod Immunol* (2014) **71**:537–42. doi:10.1111/aji.12245
44. Wu L, KewalRamani VN. Dendritic-cell interactions with HIV: infection and viral dissemination. *Nat Rev Immunol* (2006) **6**:859–68. doi:10.1038/nri1960
45. Iwasaki A. Mucosal dendritic cells. *Annu Rev Immunol* (2007) **25**:381–418. doi:10.1146/annurev.immunol.25.022106.141634
46. Carias AM, McCoombe S, McRaven M, Anderson M, Galloway N, Vandergrift N, et al. Defining the interaction of HIV-1 with the mucosal barriers of the female reproductive tract. *J Virol* (2013) **87**:11388–400. doi:10.1128/JVI.01377-13
47. Cavarelli M, Foglieni C, Rescigno M, Scarlatti G. R5 HIV-1 envelope attracts dendritic cells to cross the human intestinal epithelium and sample luminal virions via engagement of the CCR5. *EMBO Mol Med* (2013) **5**:776–94. doi:10.1002/emmm.201202232
48. Tsunetsugu-Yokota Y, Muhsen M. Development of human dendritic cells and their role in HIV infection: antiviral immunity versus HIV transmission. *Front Microbiol* (2013) **4**:178. doi:10.3389/fmicb.2013.00178
49. Dimitrov DS, Willey RL, Sato H, Chang LJ, Blumenthal R, Martin MA. Quantitation of human immunodeficiency virus type 1 infection kinetics. *J Virol* (1993) **67**:2182–90.
50. Carr JM, Hocking H, Li P, Burrell CJ. Rapid and efficient cell-to-cell transmission of human immunodeficiency virus infection from monocyte-derived macrophages to peripheral blood lymphocytes. *Virology* (1999) **265**:319–29. doi:10.1006/viro.1999.0047
51. Chen P, Hubner W, Spinelli MA, Chen BK. Predominant mode of human immunodeficiency virus transfer between T cells is mediated by sustained Env-dependent neutralization-resistant virological synapses. *J Virol* (2007) **81**:12582–95. doi:10.1128/JVI.00381-07
52. Sourisseau M, Sol-Foulon N, Porrot F, Blanchet F, Schwartz O. Inefficient human immunodeficiency virus replication in mobile lymphocytes. *J Virol* (2007) **81**:1000–12. doi:10.1128/JVI.01629-06
53. Martin N, Welsch S, Jolly C, Briggs JA, Vaux D, Sattentau QJ. Virological synapse-mediated spread of human immunodeficiency virus type 1 between T cells is sensitive to entry inhibition. *J Virol* (2010) **84**:3516–27. doi:10.1128/JVI.02651-09
54. Zhong P, Agosto LM, Ilinskaya A, Dorjbal B, Truong R, Derse D, et al. Cell-to-cell transmission can overcome multiple donor and target cell barriers imposed on cell-free HIV. *PLoS One* (2013) **8**:e53138. doi:10.1371/journal.pone.0053138
55. Horton RE, Vidarsson G. Antibodies and their receptors: different potential roles in mucosal defense. *Front Immunol* (2013) **4**:200. doi:10.3389/fimmu.2013.00200
56. Su B, Xu K, Lederle A, Peressin M, Biedma ME, Laumond G, et al. Neutralizing antibodies inhibit HIV-1 transfer from primary dendritic cells to autologous CD4 T lymphocytes. *Blood* (2012) **120**:3708–17. doi:10.1182/blood-2012-03-418913
57. Brandtzaeg P, Johansen FE. Mucosal B cells: phenotypic characteristics, transcriptional regulation, and homing properties. *Immunol Rev* (2005) **206**:32–63. doi:10.1111/j.0105-2896.2005.00283.x
58. Cerutti A, Rescigno M. The biology of intestinal immunoglobulin A responses. *Immunity* (2008) **28**:740–50. doi:10.1016/j.immuni.2008.05.001
59. Cerutti A, Chen K, Chorny A. Immunoglobulin responses at the mucosal interface. *Annu Rev Immunol* (2011) **29**:273–93. doi:10.1146/annurev-immunol-031210-101317
60. Chang SY, Ko HJ, Kweon MN. Mucosal dendritic cells shape mucosal immunity. *Exp Mol Med* (2014) **46**:e84. doi:10.1038/emm.2014.16

61. Brandtzaeg P. Function of mucosa-associated lymphoid tissue in antibody formation. *Immunol Invest* (2010) **39**:303–55. doi:10.3109/08820131003680369
62. Brandtzaeg P. Secretory IgA: designed for anti-microbial defense. *Front Immunol* (2013) **4**:222. doi:10.3389/fimmu.2013.00222
63. Agnello D, Denimal D, Lavaux A, Blondeau-Germe L, Lu B, Gerard NP, et al. Intrarectal immunization and IgA antibody-secreting cell homing to the small intestine. *J Immunol* (2013) **190**:4836–47. doi:10.4049/jimmunol.1202979
64. Cerutti A, Cols M, Puga I. Marginal zone B cells: virtues of innate-like antibody-producing lymphocytes. *Nat Rev Immunol* (2013) **13**:118–32. doi:10.1038/nri3383
65. Baumgarth N. The double life of a B-1 cell: self-reactivity selects for protective effector functions. *Nat Rev Immunol* (2011) **11**:34–46. doi:10.1038/nri2901
66. Magri G, Miyajima M, Bascones S, Mortha A, Puga I, Cassis L, et al. Innate lymphoid cells integrate stromal and immunological signals to enhance antibody production by splenic marginal zone B cells. *Nat Immunol* (2014) **15**:354–64. doi:10.1038/ni.2830
67. Cerutti A. The regulation of IgA class switching. *Nat Rev Immunol* (2008) **8**:421–34. doi:10.1038/nri2322
68. Levesque MC, Moody MA, Hwang KK, Marshall DJ, Whitesides JF, Amos JD, et al. Polyclonal B cell differentiation and loss of gastrointestinal tract germinal centers in the earliest stages of HIV-1 infection. *PLoS Med* (2009) **6**:e1000107. doi:10.1371/journal.pmed.1000107
69. Haynes BF, Liao HX, Tomaras GD. Is developing an HIV-1 vaccine possible? *Curr Opin HIV AIDS* (2010) **5**:362–7. doi:10.1097/COH.0b013e32833d2e90
70. Haynes BF, Moody MA, Liao HX, Verkoczy L, Tomaras GD. B cell responses to HIV-1 infection and vaccination: pathways to preventing infection. *Trends Mol Med* (2011) **17**:108–16. doi:10.1016/j.molmed.2010.10.008
71. Friedman J, Alam SM, Shen X, Xia SM, Stewart S, Anasti K, et al. Isolation of HIV-1-neutralizing mucosal monoclonal antibodies from human colostrum. *PLoS One* (2012) **7**:e37648. doi:10.1371/journal.pone.0037648
72. Schiffner T, Sattentau QJ, Dorrell L. Development of prophylactic vaccines against HIV-1. *Retrovirology* (2013) **10**:72. doi:10.1186/1742-4690-10-72
73. Binley JM, Wrin T, Korber B, Zwick MB, Wang M, Chappey C, et al. Comprehensive cross-clade neutralization analysis of a panel of anti-human immunodeficiency virus type 1 monoclonal antibodies. *J Virol* (2004) **78**:13232–52. doi:10.1128/JVI.78.23.13232-13252.2004
74. Polonis VR, Brown BK, Rosa Borges A, Zolla-Pazner S, Dimitrov DS, Zhang MY, et al. Recent advances in the characterization of HIV-1 neutralization assays for standardized evaluation of the antibody response to infection and vaccination. *Virology* (2008) **375**:315–20. doi:10.1016/j.virol.2008.02.007
75. Scheid JF, Mouquet H, Feldhahn N, Seaman MS, Velinzon K, Pietzsch J, et al. Broad diversity of neutralizing antibodies isolated from memory B cells in HIV-infected individuals. *Nature* (2009) **458**:636–40. doi:10.1038/nature07930
76. Julien JP, Sok D, Khayat R, Lee JH, Doores KJ, Walker LM, et al. Broadly neutralizing antibody PGT121 allosterically modulates CD4 binding via recognition of the HIV-1 gp120 V3 base and multiple surrounding glycans. *PLoS Pathog* (2013) **9**:e1003342. doi:10.1371/journal.ppat.1003342
77. Xiao X, Chen W, Feng Y, Zhu Z, Prabakaran P, Wang Y, et al. Germline-like predecessors of broadly neutralizing antibodies lack measurable binding to HIV-1 envelope glycoproteins: implications for evasion of immune responses and design of vaccine immunogens. *Biochem Biophys Res Commun* (2009) **390**:404–9. doi:10.1016/j.bbrc.2009.09.029
78. Ma BJ, Alam SM, Go EP, Lu X, Desaire H, Tomaras GD, et al. Envelope deglycosylation enhances antigenicity of HIV-1 gp41 epitopes for both broad neutralizing antibodies and their unmutated ancestor antibodies. *PLoS Pathog* (2011) **7**:e1002200. doi:10.1371/journal.ppat.1002200
79. Rolland M, Edlefsen PT, Larsen BB, Tovanabutra S, Sanders-Buell E, Hertz T, et al. Increased HIV-1 vaccine efficacy against viruses with genetic signatures in Env V2. *Nature* (2012) **490**:417–20. doi:10.1038/nature11519
80. Gottardo R, Bailer RT, Korber BT, Gnanakaran S, Phillips J, Shen X, et al. Plasma IgG to linear epitopes in the V2 and V3 regions of HIV-1 gp120 correlate with a reduced risk of infection in the RV144 vaccine efficacy trial. *PLoS One* (2013) **8**:e75665. doi:10.1371/journal.pone.0075665
81. Zolla-Pazner S, Decamp AC, Cardozo T, Karasavvas N, Gottardo R, Williams C, et al. Analysis of V2 antibody responses induced in vaccinees in the ALVAC/AIDSVAX HIV-1 vaccine efficacy trial. *PLoS One* (2013) **8**:e53629. doi:10.1371/journal.pone.0053629
82. Yates NL, Liao HX, Fong Y, Decamp A, Vandergrift NA, Williams WT, et al. Vaccine-induced Env V1-V2 IgG3 correlates with lower HIV-1 infection risk and declines soon after vaccination. *Sci Transl Med* (2014) **6**:228–39. doi:10.1126/scitranslmed.3007730
83. Bonsignori M, Pollara J, Moody MA, Alpert MD, Chen X, Hwang KK, et al. Antibody-dependent cellular cytotoxicity-mediating antibodies from an HIV-1 vaccine efficacy trial target multiple epitopes and preferentially use the VH1 gene family. *J Virol* (2012) **86**:11521–32. doi:10.1128/JVI.01023-12
84. Holl V, Hemmerter S, Burrer R, Schmidt S, Bohbot A, Aubertin AM, et al. Involvement of Fc gamma RI (CD64) in the mechanism of HIV-1 inhibition by polyclonal IgG purified from infected patients in cultured monocyte-derived macrophages. *J Immunol* (2004) **173**:6274–83. doi:10.4049/jimmunol.173.10.6274
85. Holl V, Peressin M, Decoville T, Schmidt S, Zolla-Pazner S, Aubertin AM, et al. Nonneutralizing antibodies are able to inhibit human immunodeficiency virus type 1 replication in macrophages and immature dendritic cells. *J Virol* (2006) **80**:6177–81. doi:10.1128/JVI.02625-05
86. Holl V, Peressin M, Schmidt S, Decoville T, Zolla-Pazner S, Aubertin AM, et al. Efficient inhibition of HIV-1 replication in human immature monocyte-derived dendritic cells by purified anti-HIV-1 IgG without induction of maturation. *Blood* (2006) **107**:4466–74. doi:10.1182/blood-2005-08-3490
87. Peressin M, Holl V, Schmidt S, Decoville T, Mirisky D, Lederle A, et al. HIV-1 replication in Langerhans and interstitial dendritic cells is inhibited by neutralizing and Fc-mediated inhibitory antibodies. *J Virol* (2011) **85**:1077–85. doi:10.1128/JVI.01619-10
88. Ackerman ME, Alter G. Opportunities to exploit non-neutralizing HIV-specific antibody activity. *Curr HIV Res* (2013) **11**:365–77. doi:10.2174/1570162X113116660058
89. Ackerman ME, Dugast AS, McAndrew EG, Tsoukas S, Licht AF, Irvine DJ, et al. Enhanced phagocytic activity of HIV-specific antibodies correlates with natural production of immunoglobulins with skewed affinity for FcgammaR2a and FcgammaR2b. *J Virol* (2013) **87**:5468–76. doi:10.1128/JVI.03403-12
90. Peressin M, Proust A, Schmidt S, Su B, Lambotin M, Biedma ME, et al. Efficient transfer of HIV-1 in trans and in cis from Langerhans dendritic cells and macrophages to autologous T lymphocytes. *AIDS* (2014) **28**:667–77. doi:10.1097/QAD.0000000000000193
91. Perez LG, Costa MR, Todd CA, Haynes BF, Montefiori DC. Utilization of immunoglobulin G Fc receptors by human immunodeficiency virus type 1: a specific role for antibodies against the membrane-proximal external region of gp41. *J Virol* (2009) **83**:7397–410. doi:10.1128/JVI.00656-09
92. Perez LG, Zolla-Pazner S, Montefiori DC. Antibody-dependent, Fc gamma RI-mediated neutralization of HIV-1 in TZM-bl cells occurs independently of phagocytosis. *J Virol* (2013) **87**:5287–90. doi:10.1128/JVI.00278-13
93. Holl V, Peressin M, Moog C. Antibody-mediated Fcgamma receptor-based mechanisms of HIV inhibition: recent findings and new vaccination strategies. *Viruses* (2009) **1**:1265–94. doi:10.3390/v1031265
94. Ackerman ME, Moldt B, Wyatt RT, Dugast AS, McAndrew E, Tsoukas S, et al. A robust, high-throughput assay to determine the phagocytic activity of clinical antibody samples. *J Immunol Methods* (2011) **366**:8–19. doi:10.1016/j.jim.2010.12.016
95. Forthal D, Hope TJ, Alter G. New paradigms for functional HIV-specific non-neutralizing antibodies. *Curr Opin HIV AIDS* (2013) **8**:392–400. doi:10.1097/COH.0b013e328363d486
96. Lewis GK. Qualitative and quantitative variables that affect the potency of Fc-mediated effector function in vitro and in vivo: considerations for passive immunization using non-neutralizing antibodies. *Curr HIV Res* (2013) **11**:354–64. doi:10.2174/1570162X113116660060
97. DeVico AL, Gallo RC. Control of HIV-1 infection by soluble factors of the immune response. *Nat Rev Microbiol* (2004) **2**:401–13. doi:10.1038/nrmicro878
98. Moody MA, Liao HX, Alam SM, Scearce RM, Plonk MK, Kozink DM, et al. Anti-phospholipid human monoclonal antibodies inhibit CCR5-tropic HIV-1 and induce beta-chemokines. *J Exp Med* (2010) **207**:763–76. doi:10.1084/jem.20091281
99. Chung AW, Ghebremichael M, Robinson H, Brown E, Choi I, Lane S, et al. Polyfunctional Fc-effector profiles mediated by IgG subclass selection distinguish RV144 and VAX003 vaccines. *Sci Transl Med* (2014) **6**:228–38. doi:10.1126/scitranslmed.3007736

100. Lyerly HK, Matthews TJ, Langlois AJ, Bolognesi DP, Weinhold KJ. Human T-cell lymphotropic virus IIIB glycoprotein (gp120) bound to CD4 determinants on normal lymphocytes and expressed by infected cells serves as target for immune attack. *Proc Natl Acad Sci U S A* (1987) **84**:4601–5. doi:10.1073/pnas.84.13.4601
101. Forthal DN, Moog C. Fc receptor-mediated antiviral antibodies. *Curr Opin HIV AIDS* (2009) **4**:388–93. doi:10.1097/COH.0b013e32832f0a89
102. Trinchieri G, Valiante N. Receptors for the Fc fragment of IgG on natural killer cells. *Nat Immun* (1993) **12**:218–34.
103. Ahmad A, Menezes J. Antibody-dependent cellular cytotoxicity in HIV infections. *FASEB J* (1996) **10**:258–66.
104. Alter G, Altfeld M. NK cells in HIV-1 infection: evidence for their role in the control of HIV-1 infection. *J Intern Med* (2009) **265**:29–42. doi:10.1111/j.1365-2796.2008.02045.x
105. Berger CT, Alter G. Natural killer cells in spontaneous control of HIV infection. *Curr Opin HIV AIDS* (2011) **6**:208–13. doi:10.1097/COH.0b013e3283457798
106. Smalls-Mantey A, Connors M, Sattentau QJ. Comparative efficiency of HIV-1-infected T cell killing by NK cells, monocytes and neutrophils. *PLoS One* (2013) **8**:e74858. doi:10.1371/journal.pone.0074858
107. Lambotte O, Ferrari G, Moog C, Yates NL, Liao HX, Parks RJ, et al. Heterogeneous neutralizing antibody and antibody-dependent cell cytotoxicity responses in HIV-1 elite controllers. *AIDS* (2009) **23**:897–906. doi:10.1097/QAD.0b013e328329f97d
108. Lambotte O, Pollara J, Boufassa F, Moog C, Venet A, Haynes BF, et al. High antibody-dependent cellular cytotoxicity responses are correlated with strong CD8 T cell viral suppressive activity but not with B57 status in HIV-1 elite controllers. *PLoS One* (2013) **8**:e74855. doi:10.1371/journal.pone.0074855
109. Gomez-Roman VR, Patterson LJ, Venzon D, Liewehr D, Aldrich K, Florese R, et al. Vaccine-elicited antibodies mediate antibody-dependent cellular cytotoxicity correlated with significantly reduced acute viremia in rhesus macaques challenged with SIVmac251. *J Immunol* (2005) **174**:2185–9. doi:10.4049/jimmunol.174.4.2185
110. Hessell AJ, Hangartner L, Hunter M, Havenith CEG, Beurskens FJ, Bakker JM, et al. Fc receptor but not complement binding is important in antibody protection against HIV. *Nature* (2007) **449**:101–U175. doi:10.1038/nature06106
111. Yates NL, Stacey AR, Nolen TL, Vandergrift NA, Moody MA, Montefiori DC, et al. HIV-1 gp41 envelope IgA is frequently elicited after transmission but has an initial short response half-life. *Mucosal Immunol* (2013) **6**:692–703. doi:10.1038/mi.2012.107
112. Mestecky J, Jackson S, Moldoveanu Z, Nesbit LR, Kulhavy R, Prince SJ, et al. Paucity of antigen-specific IgA responses in sera and external secretions of HIV-type 1-infected individuals. *AIDS Res Hum Retroviruses* (2004) **20**:972–88. doi:10.1089/aid.2004.20.972
113. Mazzoli S, Trabattoni D, Lo Caputo S, Piconi S, Ble C, Meacci F, et al. HIV-specific mucosal and cellular immunity in HIV-seronegative partners of HIV-seropositive individuals. *Nat Med* (1997) **3**:1250–7. doi:10.1038/nm1197-1250
114. Broliden K, Hinkula J, Devito C, Kiama P, Kimani J, Trabbatoni D, et al. Functional HIV-1 specific IgA antibodies in HIV-1 exposed, persistently IgG seronegative female sex workers. *Immunol Lett* (2001) **79**:29–36. doi:10.1016/S0165-2478(01)00263-2
115. Tudor D, Derrien M, Diomede L, Drillet AS, Houimel M, Moog C, et al. HIV-1 gp41-specific monoclonal mucosal IgAs derived from highly exposed but IgG-seronegative individuals block HIV-1 epithelial transcytosis and neutralize CD4(+) cell infection: an IgA gene and functional analysis. *Mucosal Immunol* (2009) **2**:412–26. doi:10.1038/mi.2009.89
116. Mantis NJ, Palaia J, Hessell AJ, Mehta S, Zhu Z, Corthesy B, et al. Inhibition of HIV-1 infectivity and epithelial cell transfer by human monoclonal IgG and IgA antibodies carrying the b12 V region. *J Immunol* (2007) **179**:3144–52. doi:10.4049/jimmunol.179.5.3144
117. Phalipon A, Cardona A, Kraehenbuhl JP, Edelman L, Sansonetti PJ, Corthesy B. Secretory component: a new role in secretory IgA-mediated immune exclusion in vivo. *Immunity* (2002) **17**:107–15. doi:10.1016/S1074-7613(02)00341-2
118. Corthesy B. Multi-faceted functions of secretory IgA at mucosal surfaces. *Front Immunol* (2013) **4**:185. doi:10.3389/fimmu.2013.00185
119. Stieh D, Gioia C, McRaven M, Cianci G, Kiser P, Hope T. Development of an imaging based virus aggregation assay for vaccine development. *Retrovirology* (2012) **9**(Suppl 2):319. doi:10.1186/1742-4690-9-S2-P319
120. Tomaras GD, Ferrari G, Shen X, Alam SM, Liao HX, Pollara J, et al. Vaccine-induced plasma IgA specific for the C1 region of the HIV-1 envelope blocks binding and effector function of IgG. *Proc Natl Acad Sci U S A* (2013) **110**:9019–24. doi:10.1073/pnas.1301456110
121. Moris A. Antiviral treatments over cell-to-cell infection: is it just a matter of dose? *AIDS* (2013) **27**:2481–3. doi:10.1097/01.aids.0000432447.45486.03
122. Schiffner T, Sattentau QJ, Duncan CJ. Cell-to-cell spread of HIV-1 and evasion of neutralizing antibodies. *Vaccine* (2013) **31**:5789–97. doi:10.1016/j.vaccine.2013.10.020
123. Jolly C, Sattentau QJ. Retroviral spread by induction of virological synapses. *Traffic* (2004) **5**:643–50. doi:10.1111/j.1600-0854.2004.00209.x
124. Sattentau QJ. Cell-to-cell spread of retroviruses. *Viruses* (2010) **2**:1306–21. doi:10.3390/v2061306
125. Vasiliver-Shamis G, Dustin ML, Hioe CE. HIV-1 virological synapse is not simply a copycat of the immunological synapse. *Viruses* (2010) **2**:1239–60. doi:10.3390/v2051239
126. Coleman CM, Gelais CS, Wu L. Cellular and viral mechanisms of HIV-1 transmission mediated by dendritic cells. *Adv Exp Med Biol* (2013) **762**:109–30. doi:10.1007/978-1-4614-4433-6_4
127. Frankel SS, Steinman RM, Michael NL, Kim SR, Bhardwaj N, Pope M, et al. Neutralizing monoclonal antibodies block human immunodeficiency virus type 1 infection of dendritic cells and transmission to T cells. *J Virol* (1998) **72**:9788–94.
128. Ketas TJ, Frank I, Klasse PJ, Sullivan BM, Gardner JP, Spenlehauer C, et al. Human immunodeficiency virus type 1 attachment, coreceptor, and fusion inhibitors are active against both direct and trans infection of primary cells. *J Virol* (2003) **77**:2762–7. doi:10.1128/JVI.77.4.2762-2767.2003
129. Cavrois M, Neidleman J, Kreisberg JF, Greene WC. In vitro derived dendritic cells trans-infect CD4 T cells primarily with surface-bound HIV-1 virions. *PLoS Pathog* (2007) **3**:e4. doi:10.1371/journal.ppat.0030004
130. van Montfort T, Thomas AA, Pollakis G, Paxton WA. Dendritic cells preferentially transfer CXCR4-using human immunodeficiency virus type 1 variants to CD4+ T lymphocytes in trans. *J Virol* (2008) **82**:7886–96. doi:10.1128/JVI.00245-08
131. Yu HJ, Reuter M, McDonald D. HIV traffics through a specialized, surface-accessible intracellular compartment during trans-infection of T cells by mature dendritic cells. *PLoS Pathog* (2008) **4**(8):e1000134. doi:10.1371/journal.ppat.1000134
132. Abela IA, Berlinger L, Schanz M, Reynell L, Gunthard HF, Rusert P, et al. Cell-cell transmission enables HIV-1 to evade inhibition by potent CD4bs directed antibodies. *PLoS Pathog* (2012) **8**:e1002634. doi:10.1371/journal.ppat.1002634
133. Durham ND, Yewdall AW, Chen P, Lee R, Zony C, Robinson JE, et al. Neutralization resistance of virological synapse-mediated HIV-1 Infection is regulated by the gp41 cytoplasmic tail. *J Virol* (2012) **86**:7484–95. doi:10.1128/JVI.00230-12
134. Sagar M, Akiyama H, Etemad B, Ramirez N, Freitas I, Gummuluru S. Transmembrane domain membrane proximal external region but not surface unit-directed broadly neutralizing HIV-1 antibodies can restrict dendritic cell-mediated HIV-1 trans-infection. *J Infect Dis* (2012) **205**:1248–57. doi:10.1093/infdis/jis183
135. Sanders RW, De Jong EC, Baldwin CE, Schuitemaker JH, Kapsenberg ML, Berkhout B. Differential transmission of human immunodeficiency virus type 1 by distinct subsets of effector dendritic cells. *J Virol* (2002) **76**:7812–21. doi:10.1128/JVI.76.15.7812-7821.2002
136. McDonald D. Dendritic cells and HIV-1 trans-infection. *Viruses* (2010) **2**:1704–17. doi:10.3390/v2081704
137. Rodriguez-Plata MT, Puigdomenech I, Izquierdo-Useros N, Puertas MC, Carrillo J, Erkizia I, et al. The infectious synapse formed between mature dendritic cells and CD4(+) T cells is independent of the presence of the HIV-1 envelope glycoprotein. *Retrovirology* (2013) **10**:42. doi:10.1186/1742-4690-10-42
138. Duncan CJ, Williams JP, Schiffner T, Gartner K, Ochsenbauer C, Kappes J, et al. High-multiplicity HIV-1 infection and neutralizing antibody evasion mediated by the macrophage-T cell virological synapse. *J Virol* (2014) **88**:2025–34. doi:10.1128/JVI.03245-13
139. Su B, Biedma ME, Lederle A, Peressin M, Lambotin M, Proust A, et al. Dendritic cell-lymphocyte cross talk downregulates host restriction factor SAMHD1 and stimulates HIV-1 replication in dendritic cells. *J Virol* (2014) **88**:5109–21. doi:10.1128/JVI.03057-13
140. Tardif MR, Gilbert C, Thibault S, Fortin JF, Tremblay MJ. LFA-1 antagonists as agents limiting human immunodeficiency virus type 1 infection and

transmission and potentiating the effect of the fusion inhibitor T-20. *Antimicrob Agents Chemother* (2009) **53**:4656–66. doi:10.1128/AAC.00117-09
141. Kondo N, Melikyan GB. Intercellular adhesion molecule 1 promotes HIV-1 attachment but not fusion to target cells. *PLoS One* (2012) **7**:e44827. doi:10.1371/journal.pone.0044827
142. Huppa JB, Davis MM. T-cell-antigen recognition and the immunological synapse. *Nat Rev Immunol* (2003) **3**:973–83. doi:10.1038/nri1245
143. Dustin ML, Tseng SY, Varma R, Campi G. T cell-dendritic cell immunological synapses. *Curr Opin Immunol* (2006) **18**:512–6. doi:10.1016/j.coi.2006.05.017
144. Fooksman DR, Vardhana S, Vasiliver-Shamis G, Liese J, Blair DA, Waite J, et al. Functional anatomy of T cell activation and synapse formation. *Annu Rev Immunol* (2010) **28**:79–105. doi:10.1146/annurev-immunol-030409-101308
145. Hivroz C, Chemin K, Tourret M, Bohineust A. Crosstalk between T lymphocytes and dendritic cells. *Crit Rev Immunol* (2012) **32**:139–55. doi:10.1615/CritRevImmunol.v32.i2.30
146. Kulpa DA, Brehm JH, Fromentin R, Cooper A, Cooper C, Ahlers J, et al. The immunological synapse: the gateway to the HIV reservoir. *Immunol Rev* (2013) **254**:305–25. doi:10.1111/imr.12080
147. Haase AT. Population biology of HIV-1 infection: viral and CD4+ T cell demographics and dynamics in lymphatic tissues. *Annu Rev Immunol* (1999) **17**:625–56. doi:10.1146/annurev.immunol.17.1.625
148. Sloan RD, Kuhl BD, Mesplede T, Munch J, Donahue DA, Wainberg MA. Productive entry of HIV-1 during cell-to-cell transmission via dynamin-dependent endocytosis. *J Virol* (2013) **87**:8110–23. doi:10.1128/JVI.00815-13
149. Jolly C, Kashefi K, Hollinshead M, Sattentau QJ. HIV-1 cell to cell transfer across an Env-induced, actin-dependent synapse. *J Exp Med* (2004) **199**:283–93. doi:10.1084/jem.20030648
150. Haller C, Fackler OT. HIV-1 at the immunological and T-lymphocytic virological synapse. *Biol Chem* (2008) **389**:1253–60. doi:10.1515/BC.2008.143
151. Jolly C, Welsch S, Michor S, Sattentau QJ. The regulated secretory pathway in CD4 T cells contributes to human immunodeficiency virus type-1 cell-to-cell spread at the virological synapse. *PLoS Pathog* (2011) **7**:e1002226. doi:10.1371/journal.ppat.1002226
152. Dale BM, Alvarez RA, Chen BK. Mechanisms of enhanced HIV spread through T-cell virological synapses. *Immunol Rev* (2013) **251**:113–24. doi:10.1111/imr.12022
153. Hubner W, McNerney GP, Chen P, Dale BM, Gordon RE, Chuang FY, et al. Quantitative 3D video microscopy of HIV transfer across T cell virological synapses. *Science* (2009) **323**:1743–7. doi:10.1126/science.1167525
154. Massanella M, Puigdomenech I, Cabrera C, Fernandez-Figueras MT, Aucher A, Gaibelet G, et al. Antigp41 antibodies fail to block early events of virological synapses but inhibit HIV spread between T cells. *AIDS* (2009) **23**:183–8. doi:10.1097/QAD.0b013e32831ef1a3
155. Puigdomenech I, Massanella M, Cabrera C, Clotet B, Blanco J. On the steps of cell-to-cell HIV transmission between CD4 T cells. *Retrovirology* (2009) **6**:89. doi:10.1186/1742-4690-6-89
156. Dale BM, McNerney GP, Thompson DL, Hubner W, De Los Reyes K, Chuang FY, et al. Cell-to-cell transfer of HIV-1 via virological synapses leads to endosomal virion maturation that activates viral membrane fusion. *Cell Host Microbe* (2011) **10**:551–62. doi:10.1016/j.chom.2011.10.015
157. Sanchez-Palomino S, Massanella M, Carrillo J, Garcia A, Garcia F, Gonzalez N, et al. A cell-to-cell HIV transfer assay identifies humoral responses with broad neutralization activity. *Vaccine* (2011) **29**:5250–9. doi:10.1016/j.vaccine.2011.05.016
158. Malbec M, Porrot F, Rua R, Horwitz J, Klein F, Halper-Stromberg A, et al. Broadly neutralizing antibodies that inhibit HIV-1 cell to cell transmission. *J Exp Med* (2013) **210**:2813–21. doi:10.1084/jem.20131244
159. Robinson WE Jr, Montefiori DC, Mitchell WM. A human immunodeficiency virus type 1 (HIV-1) infection-enhancing factor in seropositive sera. *Biochem Biophys Res Commun* (1987) **149**:693–9. doi:10.1016/0006-291X(87)90423-2
160. Robinson WE, Montefiori DC, Mitchell WM. Antibody-dependent enhancement of human immunodeficiency virus type-1 infection. *Lancet* (1988) **1**:790–4. doi:10.1016/S0140-6736(88)91657-1
161. Wigzell H. Immunopathogenesis of HIV infection. *J Acquir Immune Defic Syndr* (1988) **1**:559–65.
162. Homsy J, Meyer M, Tateno M, Clarkson S, Levy JA. The Fc and not CD4 receptor mediates antibody enhancement of HIV infection in human cells. *Science* (1989) **244**:1357–60. doi:10.1126/science.2786647
163. van Montfort T, Nabatov AA, Geijtenbeek TB, Pollakis G, Paxton WA. Efficient capture of antibody neutralized HIV-1 by cells expressing DC-SIGN and transfer to CD4+ T lymphocytes. *J Immunol* (2007) **178**:3177–85. doi:10.4049/jimmunol.178.5.3177
164. Laurence J, Saunders A, Early E, Salmon JE. Human immunodeficiency virus infection of monocytes: relationship to Fc-gamma receptors and antibody-dependent viral enhancement. *Immunology* (1990) **70**:338–43.
165. Schutten M, Andeweg AC, Bosch ML, Osterhaus AD. Enhancement of infectivity of a non-syncytium inducing HIV-1 by sCD4 and by human antibodies that neutralize syncytium inducing HIV-1. *Scand J Immunol* (1995) **41**:18–22. doi:10.1111/j.1365-3083.1995.tb03528.x
166. Sullivan N, Sun Y, Binley J, Lee J, Barbas CF III, Parren PW, et al. Determinants of human immunodeficiency virus type 1 envelope glycoprotein activation by soluble CD4 and monoclonal antibodies. *J Virol* (1998) **72**:6332–8.
167. Baan E, De Ronde A, Stax M, Sanders RW, Luchters S, Vyankandondera J, et al. HIV-1 autologous antibody neutralization associates with mother to child transmission. *PLoS One* (2013) **8**:e69274. doi:10.1371/journal.pone.0069274
168. Gupta S, Gach JS, Becerra JC, Phan TB, Pudney J, Moldoveanu Z, et al. The neonatal Fc receptor (FcRn) enhances human immunodeficiency virus type 1 (HIV-1) transcytosis across epithelial cells. *PLoS Pathog* (2013) **9**:e1003776. doi:10.1371/journal.ppat.1003776
169. Montefiori DC, Karnasuta C, Huang Y, Ahmed H, Gilbert P, De Souza MS, et al. Magnitude and breadth of the neutralizing antibody response in the RV144 and Vax003 HIV-1 vaccine efficacy trials. *J Infect Dis* (2012) **206**:431–41. doi:10.1093/infdis/jis367
170. Francis DP, Heyward WL, Popovic V, Orozco-Cronin P, Orelind K, Gee C, et al. Candidate HIV/AIDS vaccines: lessons learned from the World's first phase III efficacy trials. *AIDS* (2003) **17**:147–56. doi:10.1097/00002030-200301240-00003
171. Forthal DN, Gabriel EE, Wang A, Landucci G, Phan TB. Association of Fcgamma receptor IIIa genotype with the rate of HIV infection after gp120 vaccination. *Blood* (2012) **120**:2836–42. doi:10.1182/blood-2012-05-431361
172. Mascola JR, Stiegler G, Vancott TC, Katinger H, Carpenter CB, Hanson CE, et al. Protection of macaques against vaginal transmission of a pathogenic HIV-1/SIV chimeric virus by passive infusion of neutralizing antibodies. *Nat Med* (2000) **6**:207–10. doi:10.1038/72318
173. Veazey RS, Shattock RJ, Pope M, Kirijan JC, Jones J, Hu Q, et al. Prevention of virus transmission to macaque monkeys by a vaginally applied monoclonal antibody to HIV-1 gp120. *Nat Med* (2003) **9**:343–6. doi:10.1038/nm833
174. Hessell AJ, Poignard P, Hunter M, Hangartner L, Tehrani DM, Bleeker WK, et al. Effective, low-titer antibody protection against low-dose repeated mucosal SHIV challenge in macaques. *Nat Med* (2009) **15**:951–4. doi:10.1038/nm.1974
175. Dereuddre-Bosquet N, Morellato-Castillo L, Brouwers J, Augustijns P, Bouchemal K, Ponchel G, et al. MiniCD4 microbicide prevents HIV infection of human mucosal explants and vaginal transmission of SHIV(162P3) in cynomolgus macaques. *PLoS Pathog* (2012) **8**:e1003071. doi:10.1371/journal.ppat.1003071
176. Hessell AJ, Epson E, Moldt B, Rakasz E, Pandey S, Sutton WF, et al. Biodistribution of neutralizing monoclonal antibodies IgG1 b12 and LALA in mucosal and lymphatic tissues of rhesus macaques. *Retrovirology* (2012) **9**(Suppl 2):204. doi:10.1186/1742-4690-9-S2-P204
177. Jaworski JP, Kobie J, Brower Z, Malherbe DC, Landucci G, Sutton WF, et al. Neutralizing polyclonal IgG present during acute infection prevents rapid disease onset in SHIVSF162P3-infected infant rhesus macaques. *J Virol* (2013) **87**:10447–59. doi:10.1128/JVI.00049-13
178. Klein K, Veazey RS, Warrier R, Hraber P, Doyle-Meyers LA, Buffa V, et al. Neutralizing IgG at the portal of infection mediates protection against vaginal simian/human immunodeficiency virus challenge. *J Virol* (2013) **87**:11604–16. doi:10.1128/JVI.01361-13
179. Hidajat R, Xiao P, Zhou Q, Venzon D, Summers LE, Kalyanaraman VS, et al. Correlation of vaccine-elicited systemic and mucosal nonneutralizing antibody activities with reduced acute viremia following intrarectal simian immunodeficiency virus SIVmac251 challenge of rhesus macaques. *J Virol* (2009) **83**:791–801. doi:10.1128/JVI.01672-08
180. Mallery DL, McEwan WA, Bidgood SR, Towers GJ, Johnson CM, James LC. Antibodies mediate intracellular immunity through tripartite

motif-containing 21 (TRIM21). *Proc Natl Acad Sci U S A* (2010) **107**:19985–90. doi:10.1073/pnas.1014074107
181. McEwan WA, Mallery DL, Rhodes DA, Trowsdale J, James LC. Intracellular antibody-mediated immunity and the role of TRIM21. *Bioessays* (2011) **33**:803–9. doi:10.1002/bies.201100093
182. James LC, Keeble AH, Khan Z, Rhodes DA, Trowsdale J. Structural basis for PRYSPRY-mediated tripartite motif (TRIM) protein function. *Proc Natl Acad Sci U S A* (2007) **104**:6200–5. doi:10.1073/pnas.0609174104
183. Keeble AH, Khan Z, Forster A, James LC. TRIM21 is an IgG receptor that is structurally, thermodynamically, and kinetically conserved. *Proc Natl Acad Sci U S A* (2008) **105**:6045–50. doi:10.1073/pnas.0800159105
184. Watkinson RE, Tam JC, Vaysburd MJ, James LC. Simultaneous neutralization and innate immune detection of a replicating virus by TRIM21. *J Virol* (2013) **87**:7309–13. doi:10.1128/JVI.00647-13
185. Ngo-Giang-Huong N, Candotti D, Goubar A, Autran B, Maynart M, Sicard D, et al. HIV type 1-specific IgG2 antibodies: markers of helper T cell type 1 response and prognostic marker of long-term nonprogression. *AIDS Res Hum Retroviruses* (2001) **17**:1435–46. doi:10.1089/088922201753197105
186. French MA, Tanaskovic S, Law MG, Lim A, Fernandez S, Ward LD, et al. Vaccine-induced IgG2 anti-HIV p24 is associated with control of HIV in patients with a 'high-affinity' FcgammaRIIa genotype. *AIDS* (2010) **24**:1983–90. doi:10.1097/QAD.0b013e32833c1ce0
187. Ng CT, Jaworski JP, Jayaraman P, Sutton WF, Delio P, Kuller L, et al. Passive neutralizing antibody controls SHIV viremia and enhances B cell responses in infant macaques. *Nat Med* (2010) **16**:1117–9. doi:10.1038/nm.2233
188. Michaud HA, Gomard T, Gros L, Thiolon K, Nasser R, Jacquet C, et al. A crucial role for infected-cell/antibody immune complexes in the enhancement of endogenous antiviral immunity by short passive immunotherapy. *PLoS Pathog* (2010) **6**:e1000948. doi:10.1371/journal.ppat.1000948
189. Abes R, Gelize E, Fridman WH, Teillaud JL. Long-lasting antitumor protection by anti-CD20 antibody through cellular immune response. *Blood* (2010) **116**:926–34. doi:10.1182/blood-2009-10-248609
190. Moldt B, Schultz N, Dunlop DC, Alpert MD, Harvey JD, Evans DT, et al. A panel of IgG1 b12 variants with selectively diminished or enhanced affinity for Fc gamma receptors to define the role of effector functions in protection against HIV. *J Virol* (2011) **85**:10572–81. doi:10.1128/JVI.05541-11
191. Moldt B, Shibata-Koyama M, Rakasz EG, Schultz N, Kanda Y, Dunlop DC, et al. A nonfucosylated variant of the anti-HIV-1 monoclonal antibody b12 has enhanced Fc gamma RIIIa-mediated antiviral activity in vitro but does not improve protection against mucosal SHIV challenge in macaques. *J Virol* (2012) **86**:6189–96. doi:10.1128/JVI.00491-12
192. Ruffin N, Borggren M, Euler Z, Fiorino F, Grupping K, Hallengard D, et al. Rational design of HIV vaccines and microbicides: report of the EUROPRISE annual conference 2011. *J Transl Med* (2012) **10**:144. doi:10.1186/1479-5876-10-144
193. PX'Therapeutics. *PX'Therapeutics Annonce l'essai Clinique EuroNeut-41 Portant sur un Vaccin Prophylactique Contre le VIH*. Grenoble: BusinessWire (2012) 12 Mars.

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Received: 11 April 2014; accepted: 03 June 2014; published online: 18 June 2014.
*Citation: Su B and Moog C (2014) Which antibody functions are important for an HIV vaccine? Front. Immunol. **5**:289. doi: 10.3389/fimmu.2014.00289*
This article was submitted to HIV and AIDS, a section of the journal Frontiers in Immunology.

frontiers in
IMMUNOLOGY

ORIGINAL RESEARCH ARTICLE
published: 30 June 2014
doi: 10.3389/fimmu.2014.00297

Mucosal SIV vaccines comprising inactivated virus particles and bacterial adjuvants induce CD8$^+$ T-regulatory cells that suppress SIV-positive CD4$^+$ T-cell activation and prevent SIV infection in the macaque model

Jean-Marie Andrieu*[1]*, *Song Chen*[2], *Chunhui Lai*[2], *Weizhong Guo*[2] *and Wei Lu*[1]

[1] *Institut de Recherches sur les Vaccins et l'Immunothérapie des Cancers et du SIDA, Centre Universitaire des Saints Peres, Université de Paris-Descartes, Paris, France*
[2] *Tropical Medicine Institute, Guangzhou University of Chinese Medicine, Guangzhou, China*

Edited by:
Marc H. V. Van Regenmortel, ESBS, CNRS, University of Strasbourg, France

Reviewed by:
Marc H. V. Van Regenmortel, ESBS, CNRS, University of Strasbourg, France
Jose Esparza, University of Maryland School of Medicine, USA

***Correspondence:**
Jean-Marie Andrieu and Wei Lu, 45 Rue des Saints Peres, Paris, France
e-mail: jean-marie.andrieu@parisdescartes.fr; louis.luwei@ird.fr

A new paradigm of mucosal vaccination against human immunodeficiency virus (HIV) infection has been investigated in the macaque model. A vaccine consisting of inactivated simian immunodeficiency virus (SIV)mac239 particles together with a living bacterial adjuvant (either the Calmette and Guerin bacillus, *Lactobacillus plantarum* or *Lactobacillus rhamnosus*) was administered to macaques via the vaginal or oral/intragastric route. In contrast to all established human and veterinary vaccines, these three vaccine regimens did not elicit SIV-specific antibodies nor cytotoxic T-lymphocytes but induced a previously unrecognized population of non-cytolytic MHCIb/E-restricted CD8$^+$ T-regulatory cells that suppressed the activation of SIV-positive CD4$^+$ T-lymphocytes. SIV reverse transcription was thereby blocked in inactivated CD4$^+$ T-cells; the initial burst of virus replication was prevented and the vaccinated macaques were protected from a challenge infection. For 3–14 months after intragastric immunization, 24 macaques were challenged intrarectally with a high dose of SIVmac239 or with the heterologous strain SIV B670 (both strains grown on macaques PBMC). Twenty-three of these animals were found to be protected for up to 48 months while all 24 control macaques became infected. This protective effect against SIV challenge together with the concomitant identification of a robust *ex vivo* correlate of protection suggests a new approach for developing an HIV vaccine in humans. The induction of this new class of CD8$^+$ T-regulatory cells could also possibly be used therapeutically for suppressing HIV replication in infected patients and this novel tolerogenic vaccine paradigm may have potential applications for treating a wide range of immune disorders and is likely to may have profound implications across immunology generally.

Keywords: inactivated SIV, bacterial adjuvants, BCG, lactobacilli, SIV replication, SIV vaccine, HIV vaccine, HIV tolerogenic vaccine

INTRODUCTION

Since the first vaccination against the small pox virus by Edward Jenner in 1796, all efficient vaccines against a viral infection have been shown to elicit virus-specific neutralizing antibodies and sometimes also cytotoxic T-lymphocytes (CTL) that prevent virus infection or eradicate the virus rapidly after it enters the body (1). So far, however, this process seems not to work for the human immunodeficiency virus (HIV) type 1 infection since, despite the tremendous advances in immunology and molecular biology accomplished since HIV discovery in 1983 (2), results of vaccine trials against HIV have indeed remained extremely poor (3). Only one trial out of more than one hundred showed a modest and short-lasting protection (4) while all the others did not induce any protective immunity (antibodies or CTL) against the virus including sadly the latest three randomized vaccine trials where more volunteers were infected in the vaccine arm than in the placebo arm (5–7); combining data from the three studies, there was an overall hazard ratio of 1.33 ($P < 0.01$) associated with vaccination (8).

For the last 30 years, our group tried to suppress HIV replication and fight HIV infection by pursuing several alternative immunological approaches. As early as 1986, we speculated that T4-cell activation (whether triggered by the virus, viral proteins, or by any other stimulus) was an absolute requirement for significant viral replication (9). This suspicion, which is now commonly accepted by scientists who study the pathogenesis of simian immunodeficiency virus (SIV) in macaques and of HIV in humans (8, 10, 11) suggested to us that it might be possible to inhibit virus replication by suppressing CD4$^+$ T-cell activation (9). We tested the possibility that viral replication might be controlled by administering the immunosuppressive drugs cyclosporine or prednisolone to infected patients. Both drugs were found to stabilize or increase CD4$^+$ T-cell counts in HIV-infected patients (12–15), probably by preventing activation-induced apoptosis of non-infected CD4$^+$ T-cells (16), but they did not control HIV-1 replication.

Since it was not possible to control viral replication by systemic non-specific immune suppression, we then explored the capacity

of dendritic cells loaded with *ex vivo*-inactivated virus to stimulate HIV-specific cellular immunity (17, 18). Using this approach, we were able to demonstrate that a therapeutic vaccine based on inactivated HIV-loaded dendritic cells had a favorable impact on HIV replication (19), a finding that was recently confirmed in HIV-infected patients who had interrupted their antiviral therapy (20). However, we did not pursue this project because the preparation of dendritic cell-based vaccine was cumbersome and expensive and was incompatible with a large scale use.

Instead of activating dendritic cells by loading them *ex vivo* with inactivated HIV, we then investigated possibility of developing a prophylactic anti-SIV vaccine by directly stimulating mucosal dendritic cells *in vivo*. We describe here the attempts we made during the last 10 years to develop prophylactic mucosal vaccines using inactivated SIV mac239 (iSIV) adjuvanted by non-pathogenic living bacteria potentially able to cooperate with mucosal dendritic cells. These experiments were performed in macaques of Chinese origin, which best mimics HIV infection in humans (21, 22).

MATERIALS AND METHODS

ANIMALS

Colony-bred rhesus macaques (*Macaca mulatta*) of Chinese origin were housed in accordance with the regulations of the National Institutes of Health "Guide for the Care and Use of Laboratory Animals." The committee for animal studies of the Tropical Medicine Institute, Guangzhou, University of Chinese Medicine, has approved the study and confirmed that it has been done appropriately. All animals were in good health, 2–4 years old, weighed 4–6 kg, and were seronegative for SIV, simian retrovirus, simian T-cells lymphotropic virus 1, hepatitis B virus, and Herpes virus simiae.

VACCINE PREPARATION

The vaccines used throughout this study were a mixture of two components: the iSIV and a bacterial adjuvant, which was either the Bacillus of Calmette & Guerin (BCG), the *Lactobacillus plantarum* (LP), or the *Lactobacillus rhamnosus* (LR).

Bacterial preparations

Three bacterial preparations were used: the BCG, the LP, and the LR. The BCG (strain SSI 1331, Sanofi-Pasteur) was used at a final dose of 5×10^6 or 7.5×10^7 cfu for each (intravaginal or intragastrical) vaccination or boost. Each dose of vaccine was freshly prepared in 1 mL of RPMI. The LP (ATCC8014) was cultivated at 37°C in MRS medium with a rotation rate of 200 rpm until reaching a final LP concentration of around 10^{10} cfu/ml (i.e., an optical density of 1.0 at 600 nm). The LR (lcr35, Probionov, 15000 Aurillac, France) was received in lyophilized form at a concentration of around 1×10^{11} cfu/g.

Inactivated virus preparation

The virus production was performed in CEM174 cells inoculated with SIVmac239 [see Lu et al. (23)]. Culture supernatants were collected at peak viral production. In order to check what could be the simplest and safest inactivation system, we tested three different modalities: when associated with the BCG, the virus was inactivated with 250 μM aldrithiol-2 (AT-2) in the same manner as we did previously (18); when associated with LP, the virus was inactivated with AT-2 and then by heat (56°C for 30 min) [see Lu et al. (23)]; finally, when associated with LR, the virus was inactivated in the simplest as possible manner, by heat (56°C for 30 min) twice at 30 min-interval. The iSIV was inoculated to CEM174 cells to verify the 100% inhibition of viral infectivity.

DELIVERY OF THE VACCINES

Monkeys were anesthetized with tiletamine hydrochloride and intramuscular zolazepam (0.7 mg/kg) (after having fasted overnight for those which were immunized via the intragastric route).

Intravaginal immunization with iSIV + BCG

Monkeys were administered intravaginally 5 ml of a vaccine comprising 10^9 particles of iSIV and 5×10^6 cfu of BCG (strain SSI 1331). A booster intravaginal immunization with the same dose was applied 2 months later. Control animals remained naïve until challenge.

Intragastric immunization with iSIV + BCG

Monkeys after having fasted overnight were anesthetized. They were administrated intragastrically with 25 ml of PBS buffer and half an hour later with 5 ml of a viral–bacterial preparation containing 10^9 copies/ml of iSIV and 1.5×10^7 cfu/ml of BCG in maltodextrin (20%) solution. A booster intravaginal immunization with the same dose was applied 2 months later. Control animals received the same doses of BCG alone according to the same protocol.

Intragastric immunization with iSIV + LP or LR

The macaques were administrated intragastrically 30 ml of a first preparation containing 4×10^7 copies/ml of iSIV and 3×10^9 cfu/ml of LP or LR in maltodextrin (20%) solution. Monkeys then received intragastrically 25 ml of the same preparation each 30 min for 3 h; the same protocol was performed over five consecutive days. Overall, each macaque received a total of 3.5×10^{10} copies of iSIV and 2.6×10^{12} cfu of LP or LR. Control animals received the same doses of LP alone, LR alone, or iSIV according to the same protocol.

Overall, 37 macaques were vaccinated by one of the above-mentioned protocols and 45 animals served as control.

SIV SUPPRESSION ASSAY: *EX VIVO* ANTIVIRAL ACTIVITY OF VACCINE-INDUCED CD8$^+$ T-CELLS

Autologous CD4$^+$ T-cells from each animal purified by magnetic positive-labeling (MicroBeads, Miltenyi Biotec) were acutely infected with SIVmac239 (10^{-3} multiplicity of infection) in the presence or the absence of magnetically purified CD8$^+$ T-cells at a CD4/CD8 ratio of 1:3 and then stimulated with staphylococcal enterotoxin B (SEB) and anti-CD3/anti-CD28 antibodies for 16 h. After washing, the cells were cultivated in quadruplicates in 96-well plates. Cultures were maintained in a final volume of 200 μl per well of RPMI 1640 medium containing 100 IU of human rIL2 (Roche Diagnostics GmbH, Mannheim, Germany) for 5 days. Viral loads were measured by a real-time RT-PCR in culture supernatants collected at day 5 [see Lu et al. (23)]. *Ex vivo*

antiviral activity was the ratio of geometric means of viral concentration in the culture supernatants from the infected $CD4^+$ target cells only over the geometric means of viral concentration in the supernatants from the mixed $CD8^+$ and $CD4^+$ T-cells.

VIRAL CHALLENGES

For 3–14 months after the administration of the vaccine or the controls, vaccinated and control animals were inoculated intravenously with 50 MID_{100} (200 $TCID_{50}$) or intrarectally with 2,500 MID_{100} (100,000 $TCID_{50}$) of pathogenic SIVmac239 (cultivated on macaques PBMC). Monkeys were rechallenged by intravenous or intrarectal route with the same high doses of infectious SIVmac239. Eight macaques were intrarectally rechallenged with 100,000 $TCID_{50}$ of pathogenic SIVB670 (a distinct strain of SIV provided by F. Villinger, Emory University School of Medicine, Atlanta, Georgia). SIVB670 was propagated *in vitro* using macaque PBMC and the first passages of the original viral stocks were used for challenge.

OTHER METHODS

For details on other methods used in this study (MHC class I typing, assay for antibody responses to SIV, flow cytometry, SIV-specific cell proliferation assay, SIV-specific ELISPOT assay, T-cell suppression assay, T-cell cytotoxicity assay, viral loads measurements, depletion of $CD8^+$ T-cells *in vivo*, and statistical analysis), see Section "Experimental Procedures" in Lu et al. (23).

RESULTS

MUCOSAL VACCINATION WITH INACTIVATED SIV AND BCG

We first tested a vaccine including iSIV as immunogen and the BCG as a bacterial adjuvant (24). We chose the BCG because it had been shown to interact with dendritic cells (25, 26) as well as to stimulate cellular immunity in some types of cancers (27, 28). Moreover, BCG had been given orally to several millions of people in Brazil until 1974 without serious side effects (29).

Intravaginal immunization followed by intravenous challenge

Six macaques were administered intravaginally 5 ml of a vaccine comprising 10^9 particles of iSIV and 5×10^6 cfu of BCG (strain SSI 1331). A booster intravaginal immunization was applied 2 months later. Two months later (i.e., 4 months after the initial vaccination), the six animals were challenged by intravenous route with 200 $TCID_{50}$ of SIVmac239. Simultaneously five vaccine-free control animals were also challenged with the same dose of SIVmac239. Al control macaques showed a typical primary infection with plasma viral loads peaking at 10^6–10^7 viral copies/ml between days 10 and 14 post-challenge and then remaining high ($>10^5$ copies/ml) over the next 60 days. They also developed high levels of PBMC SIV DNA and became seropositive with high titers of SIV antibodies. Among the six animals that received the intravaginal vaccine, plasma viral RNA loads of two of them, after the usual peak ($>10^6$ copies/ml), decreased to lower set-point ($<10^3$ copies/ml) than normal whereas their SIV DNA remained $\leq$1,000 copies/10^6 in PBMC or lymph node cells. More surprisingly, the four remaining macaques showed very low viral RNA and DNA peaks ($\leq 10^3$ SIV RNA copies/ml and $<10^3$ SIV DNA copies//10^6 PBMC) by days 10–14, which dropped down to undetectable/hardly detectable levels thereafter (**Figures 1A,B**).

Intravaginal immunization followed by intrarectal challenge

Seven new macaques were then administered intravaginally the same vaccine with the same boosts while five controls animals remained vaccine-free. Four months later, the 12 animals were challenged via the intrarectal route with 100,000 $TCID_{50}$ of SIVmac239. The five control animals showed the same typical primary infection as described above. Moreover, three out of the seven vaccinated animals showed also a primary infection resembling the usual one but with viral RNA and DNA load set-points significantly lower than those observed in control animals. The new information was that four out of the seven vaccinated macaques remained with undetectable levels of plasma SIV RNA (<10 SIV RNA copies/ml) and PBMC proviral DNA (<1 SIV DNA copies/10^6 PBMC) over the 60 days post-challenge (**Figures 1C,D**).

Protection against repeated intravenous or intrarectal challenges after intravaginal immunization

Among the four animals with undetectable viral loads following intravenous challenge, three were tested for their long-term capacity to fight new SIV challenges. For 2 and 8 months after their initial intravenous challenge (i.e., 6 and 12 months after their immunization), these three monkeys received a second and a third intravenous challenge with the same dose of SIV239 (200 $TCID_{50}$). After each of these viral challenges, similar low plasma RNA SIV peaks were observed at day 10 but by day 30, viral loads of all three macaques had dropped down to undetectable levels. For 21 and 27 months after initial vaccination, the three macaques were further challenged with SIVmac239, this time via the intrarectal route (100,000 $TCID_{50}$) and the three animals showed again no detectable plasma virus (<10 SIV RNA copies/ml) while their PBMC SIV DNA levels remained at the limit of positivity. Overall, viral replication of these three vaccinated macaques remained stably suppressed as long as we tested it (28 months) in spite of five SIV239 challenges (**Figure 1E**).

Oral (intragastric) immunization followed by intrarectal challenges

Four new macaques were then administered through a gastric tube 5 ml of a vaccine comprising iSIV and BCG. Vaccine administration was preceded and followed by the injection of 15 ml of 0.1 M sodium bicarbonate in the macaques gastric tube. Booster vaccinations with the same preparations were repeated at days 30 and 60. Four control animals received BCG alone according to the same protocol. An intrarectal viral challenge (100,000 $TCID_{50}$ of SIVmac239) was then given to the eight animals at day 90. The four control animals showed a typical primary infection with a plasma viral peak between days 10 and 14 post-challenge. In strong contrast, the four animals that received the vaccine were sterilely protected as shown by their plasma SIV RNA and PBMC SIV DNA loads, which remained undetectable from day 1 to 60 (**Figures 1F,G**).

In light of these unexpected mucosal vaccination successes, we tested the SIV-specific antibody and cellular responses of vaccinated macaques. Surprisingly again, SIV-specific antibodies responses in intravaginally or intragastrically vaccinated macaques were undetectable or extremely weak while all control monkeys raised anti-SIV antibodies and became seropositive. SIV

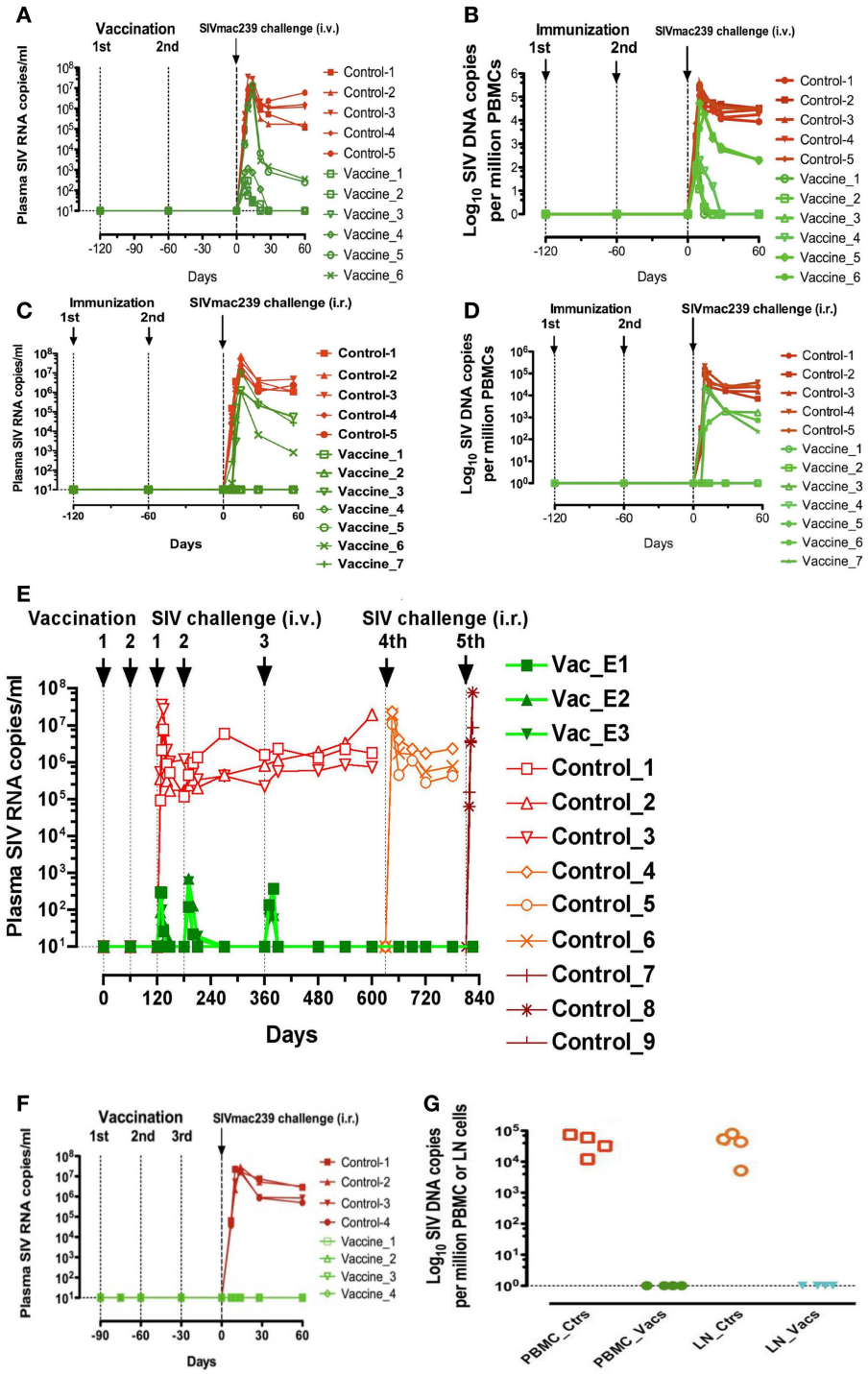


FIGURE 1 | Plasma viral loads and PBMC and lymph nodes proviral loads of rhesus macaques immunized with inactivated SIV and BCG. **(A,B)** Plasma viral loads **(A)** and PBMC proviral loads **(B)** following a challenge performed intravenously with SIVmac239 (200 TCID50) in six macaques intravaginally immunized with iSIV and BCG and five control monkeys; **(C,D)** plasma viral loads **(C)** and PBMC proviral loads **(D)** following a challenge performed intrarectally with SIVmac239 (100,000 TCID50) in seven macaques intravaginally immunized with inactivated SIV and BCG; **(E)** Plasma viral loads of three macaques intravaginally immunized with inactivated SIV and BCG following three intravenous and two intrarectal SIVmac239 challenges; **(F,G)** Plasma viral loads **(F)** and PBMC and lymph nodes proviral loads **(G)** following a challenge performed intrarectally with SIVmac239 (100,000 TCID50) in four macaques intragastrically immunized with inactivated SIV and BCG and four control monkeys immunized with inactivated BCG alone (ctrs: control, vacs: vaccinated, LN: lymph nodes).

Gag p27-specific interferon γ-releasing T-cell responses were also undetectable by ELISPOT in vaccinated macaques (data not shown).

Ex vivo antiviral activity of CD8+ T-lymphocytes in vaccinated macaques

This surprising immunovirological picture, characterized by a sterile immunity after intrarectal challenge, or complete virus replication control after intravenous challenge in the majority of mucosally vaccinated animals, in the absence of SIV-specific humoral and cellular immune responses, prompted us to examine whether SIV-specific non-conventional cellular responses could exist. For that purpose, $CD4^+$ and $CD8^+$ T-cells were purified from fresh PBMC of vaccinated or control animals. $CD4^+$ T-cells were then acutely infected with SIVmac239 and $CD4^+$ T-cells and $CD4^+$ plus $CD8^+$ T-cells were stimulated overnight with SEB plus anti-CD3 and anti-CD28 antibodies and cultivated for 5 days. The antiviral activity of the $CD8^+$ T-cells was expressed as the ratio of SIV RNA concentration in the supernatants of SIV-infected $CD4^+$ T-cell cultures in the absence of $CD8^+$ T-cells over the SIV RNA concentration in corresponding cultures in the presence of $CD8^+$ T-cells [for details, see "Method" in Ref. (23)]. Results of these assays showed that $CD8^+$ T-cells from the four out of seven intravaginally vaccinated animals that were shown later to be protected against intrarectal challenge, showed a strong antiviral activity (ratio over 100), whereas $CD8^+$ T-cells from the three animals, which were later shown not to be protected against SIV infection as well as $CD8^+$ T-cells from those which were not vaccinated had baseline levels of antiviral activity (ratio <50) (**Figure 2A**). Similarly, in the four orally vaccinated macaques, which were all found to be protected from an intrarectal challenge some weeks later, the antiviral activity was over 100 (**Figure 2B**). Altogether, in this group of vaccinated macaques, we observed a complete correlation between the pre-challenge *ex vivo* antiviral activity of $CD8^+$ T-cells and the protection observed after SIV challenge suggesting that the $CD8^+$ T-lymphocytes antiviral activity of vaccinated macaques is an excellent predictive marker of protection against SIV challenge.

Suppression of activation of SIV-specific $CD4^+$ T-cells by vaccine-induced $CD8^+$ T-cells in vaccinated macaques

Since, the initiation of replication of SIV in macaques (or HIV in humans) *in vivo*, as well as SIV (or HIV)-specific antibody and CTL induction needs SIV (or HIV)-specific $CD4^+$ T-cell activation (11), we suspected that the suppression of both SIV replication and SIV-specific immune responses observed after mucosal vaccination with iSIV plus BCG could be the consequence of the suppression of SIV-specific $CD4^+$ T-cell activation [for details on the method of $CD4^+$ T-cell activation suppression, see "Method" in Ref. (23)]. It was therefore reassuring to observe that SIV-positive $CD4^+$ T-cell activation was suppressed in the PBMC of vaccinated macaques while remaining at the high level observed in control samples when $CD8^+$ T-cells were omitted from the test sample (**Figure 2C**).

Altogether, the intravaginal co-administration of BCG and inactivated SIV protected four out of six monkeys from an intravenous SIVmac239 challenge and four out of seven monkeys from

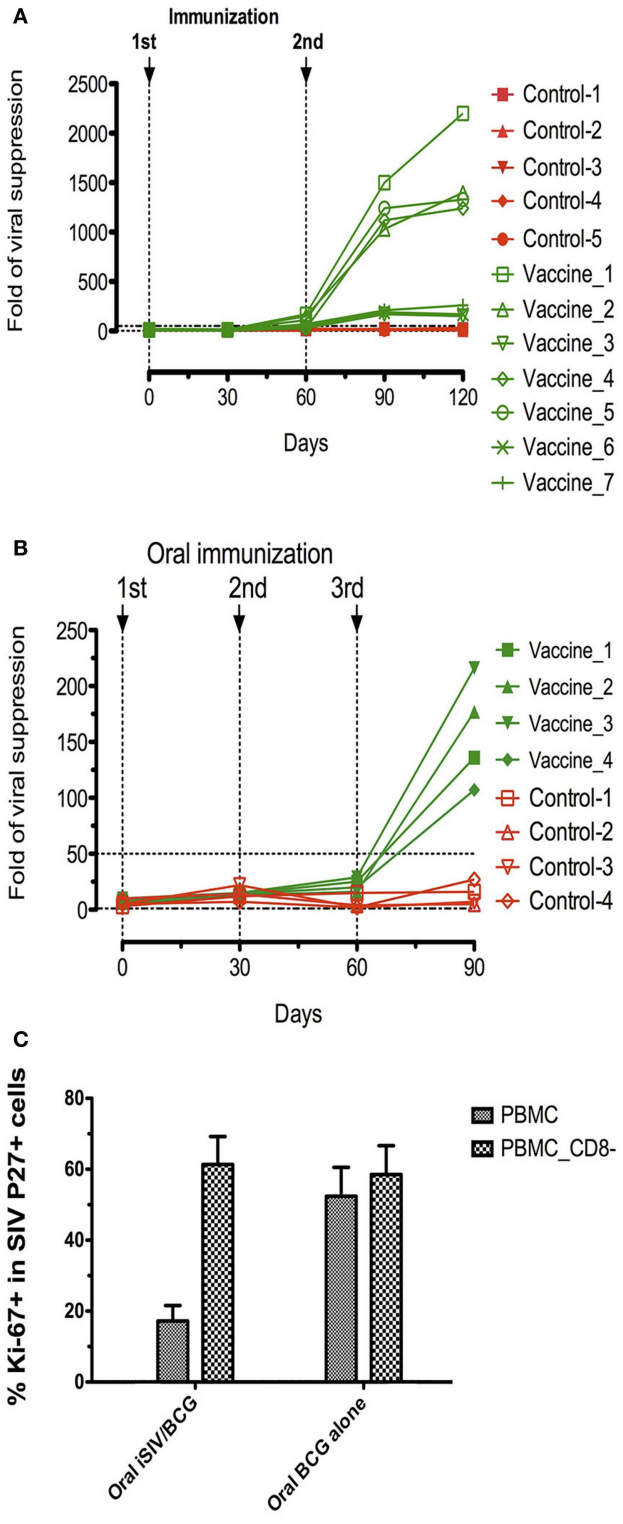


FIGURE 2 | *Ex vivo* antiviral activity and suppression of SIV positive CD4+ T-cell activation generated by vaccine-induced $CD8^+$ T-cells of rhesus macaques immunized with inactivated SIV and BCG. (A,B) *Ex vivo* antiviral activity generated by vaccine-induced $CD8^+$ T-cells in seven

(Continued)

FIGURE 2 | Continued
rhesus macaques intravaginally immunized with inactivated SIV and BCG and five control macaques **(A)** and in four rhesus macaques intragastrically immunized with inactivated SIV and BCG and four control macaques **(B)**; **(C)** percentage (mean ± SD) of the activation marker Ki-67 within SIV Gag p27$^+$ cells on the gated CD4$^+$ T-cells in AT-2 SIV-pulsed PBMC (depleted or not of CD8$^+$ T-cells) taken from animals orally immunized with inactivated SIV plus BCG or those immunized with BCG alone.

an intrarectal challenge while the intragastric administration of the same vaccine preparation protected four out of four macaques from an intrarectal challenge. Long-term protection (28 months) associated with this new type of vaccine was maintained after repeated intravenous and intrarectal challenges in the three animals where it was tested. In contrast, infection was observed in all control animals. In the four out of six macaques protected from an intravenous challenge, viral replication was completely controlled but PBMC chronically harbored the provirus; in contrast, in the 8 out 11 macaques protected from an intrarectal challenge, plasma SIV RNA and PBMC and lymph node cellular SIV DNA were both undetectable suggesting that SIV infection was blocked at entry by mucosal immunity or eradicated post-entry by systemic immunity. Surprisingly, we did not observe any SIV-specific antibodies or interferon γ-producing T-cells in the macaques that were shown to be protected after intrarectal challenge. Moreover, CD8$^+$ T-cells isolated from PBMCs of the same macaques strongly suppressed the replication of virus in acutely infected autologous CD4$^+$ T-cells *ex vivo*.

ORAL (INTRAGASTRIC) VACCINATION WITH INACTIVATED SIV AND *LACTOBACILLUS PLANTARUM*

Confirmatory studies and new information

Results observed after mucosal vaccination with BCG and iSIV were very striking, particularly because BCG, an anti-tuberculosis vaccine given worldwide for more than 90 years to more than a billion infants and children has never been even suspected to induce anergy/unresponsiveness or oral tolerance, either to its own constituents or to those of an immunogen given together. In view of these unexpected results, we decided to test an oral vaccine including iSIV and a bacterial adjuvant such as a *Lactobacillus* strain, a non-pathogenic intestinal commensal bacterium that had previously been suggested to potentially favor immune tolerance (30–32). We started by exploring the activity of an oral vaccine made of iSIV and LP (33). A group of eight macaques was immunized via the intragastric route with iSIV formulated with LP while eight control animals received LP only (four macaques) or iSIV only (four macaques). Results of this study (23) confirmed and extended several immunological and virological features already observed in iSIV + BCG-vaccinated macaques, viz: (1) both SIV-specific antibodies and interferon γ-secreting T-cells upon SIV Gag p27 stimulation were suppressed in iSIV + LP-vaccinated macaques; (2) SIV-positive CD4$^+$ T-cell activation and SIV replication in infected CD4$^+$ T-cells were also suppressed (through a non-lytic process) by the CD8$^+$ T-cells of iSIV + LP-vaccinated macaques; moreover, *ex vivo*, suppression of CD4$^+$ T-cell activation as well as virus replication suppression did not occur when CD8$^+$ T-cells were omitted from the test samples; (3) extending our previous work, we also showed that CD8$^+$ T-cells isolated from the PBMC of vaccinated macaques, when added to the test samples at a time where SIV-specific CD4$^+$ T-cells activation was already established, no longer inhibited viral replication; this new information suggested a link of causality between the suppression of SIV-specific CD4$^+$ T-cell activation and the suppression of viral replication by CD8$^+$ T-cells. We further demonstrated that CD8$^+$ T-cell-mediated antiviral activity required cell-to-cell contact and that vaccine-induced CD8$^+$ T-cells operated through a non-classical MHC-restriction mechanism that was dependent on MHC-IB/E. We also showed that classical Tregs (CD4+CD25+FoxP3+) had no role in this suppression process; (4) finally, we demonstrated that, similar to iSIV + BCG-vaccinated macaques, the eight iSIV + LP-vaccinated macaques orally were sterilely protected from high dose intrarectal challenge (100,000 $TCID_{50}$) of SIVmac239 (without any detectable level of plasma SIV RNA or PBMC proviral DNA) while the eight control animals were infected. This sterile protection was fully confirmed by the results of a second intrarectal challenge performed in four out of eight vaccinated macaques. We also showed the same virological picture as that observed in iSIV + BCG-vaccinated macaques in the four out of eight macaques, which were rechallenged via the intravenous route; a slight peak of SIV replication (≤200 DNA copies/million PBMC and 200 SIV copies/ml of plasma) was observed at day 10 post-challenge, but by day 30, plasma SIV RNA loads had dropped back to undetectable level (≤10 copies/ml) while PBMC SIV DNA were ≤10 copies/million cells showing the latent presence of SIV proviral DNA but the absence of virus replication *in vivo*. Finally, we showed that the eight iSIVmac239 + LP-vaccinated macaques (the already four intrarectally rechallenged and the already four intravenously rechallenged ones) remained fully protected against an heterologous intrarectal challenge with 100,000 $TCID_{50}$ of the antigenically distinct SIVB670, which suggested that the vaccine was cross-protective, presumably through preventing the activation of CD4$^+$ T-cells infected by another SIV strain [see Figure 4 in Ref. (23)]. Results of oral vaccinations with iSIV + BCG and iSIV + LP are summarized in **Table 1**.

Role of CD8$^+$ T-cells in protecting macaques against intrarectal SIV challenge

To better identify the *in vivo* role of CD8$^+$ T-cells in the macaques protection, the four macaques sterilely protected after three intrarectal challenge were given a fourth intrarectal challenge with 100,000 $TCID_{50}$ of SIV239; these four macaques were at the same time depleted of their CD8$^+$ T-cells from peripheral blood and lymphoid organs by a cytotoxic anti-CD8 antibody [see "Method" in Ref. (23)]. At the nadir of CD8$^+$ T-cell count (day 15), plasma viral loads of the four animals peaked around 10^4–10^6 RNA copies/ml and 10^3–10^4 copies SIV DNA/10^6 PBMC, respectively, and all animals became infected and SIV-seropositive. However, by weeks 4–7, when the CD8$^+$ T-cell levels of the four monkeys had recovered to almost normal, plasma SIV RNA and PBMC and lymph node SIV DNA dropped to baseline levels. This *in vivo* experiment demonstrated the central role of CD8$^+$ T-cells in immunized animals to prevent initial infection at the mucosal barrier. When CD8$^+$ T-cells were depleted at the moment of

Table 1 | Summary of immunovirological results observed in orally vaccinated monkeys.

Intragastric immunization	iSIV	iSIV + BCG	iSIV + LP
Production of anti-SIV antibodies	Yes	Suppressed	Suppressed
Induction of SIV-specific IFNγ-releasing T-cells	Yes	Suppressed	Suppressed
Proliferation of $CD4^+$ T-cells toward SIV antigens	NT*	NT	Suppressed
Activation of SIV^+CD4^+ T-cells	Yes	Suppressed	Suppressed
Ex-vivo suppressive $CD8^+$ Tregs	No	Yes	Yes
Sterile protection after intrarectal homologous SIV challenge	No	Yes	Yes
Sterile protection after intrarectal heterologous SIV challenge	No	NT	Yes
Absence of SIV replication after intravenous SIV challenge	No	Yes	Yes

**NT, not tested.*

the intrarectal challenge, the virus replicated freely in lymphoid organs; but as soon as the vaccine-induced $CD8^+$ T-cell population recovered, they again strongly controlled viral replication. Interestingly, recovered animals now contained SIV DNA in target cells, but without obvious viral replication (23).

Longevity of the vaccine-induced protection

To study the longevity of the vaccine protection, we immunized a new group of eight macaques with iSIV/LP in parallel with eight control macaques (four with iSIV and four with LP). We observed that seven of the eight animals that received the vaccine maintained a high *ex vivo* antiviral activity ratio (≥200) for up to 14 months while in one vaccinated macaque the antiviral activity decreased from month 12 to the baseline ratio (<100) seen in the eight control animals treated with iSIV or LP alone. By 14 months post-immunization, all 16 animals (vaccinated and controls) were challenged intrarectally with 100,000 $TCID_{50}$ of SIVmac239. Seven out of the eight iSIV/LP-immunized animals had a sterile immunity as indicated by the absence of any SIV RNA and DNA emergence in plasma, PBMC, rectal mucosa, or pelvic lymph nodes lymphocytes. Interestingly, the one vaccinated macaque, which lost its *ex vivo* antiviral activity became fully infected in the same manner as the eight control monkeys [see Figure 5 in Ref. (23); **Figures 3A,B**]. Thus, the evolution of the *ex vivo* antiviral activity of the eight vaccinated monkeys allowed us to predict, from day 360 post-immunization (i.e., 60 days before their challenge) the one out of eight monkeys that would not be protected. To control further the long-term efficacy of our vaccine, the seven protected macaques were intrarectally rechallenged by 36 months after vaccination; by 48 months post vaccination, we showed that all of them remained fully protected from infection (**Figures 3A,B**); importantly, we controlled that the repetition of viral challenges had no role in the long-term protection of the vaccinated macaques since their $CD8^+$ T-cells conserved the same high level of *ex vivo* antiviral activity before as well as after being rechallenged (data not shown).

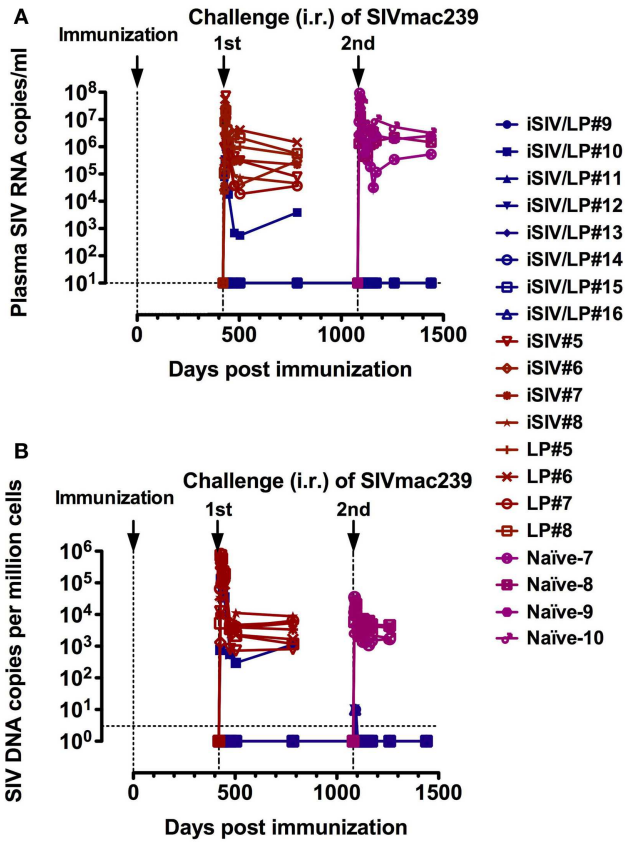


FIGURE 3 | Long-term follow-up of eight macaques intragastrically immunized with iSIV and LP and eight control monkeys immunized with iSIV alone (four macaques) or LP alone (four macaques). **(A,B)** Plasma viral loads **(A)** and PBMC proviral loads **(B)** of eight macaques submitted to two successive intrarectal challenges performed at 14 and 36 months post-immunization with SIVmac239 (100,000 TCID50).

ORAL (INTRAGASTRIC) VACCINATION WITH INACTIVATED SIV AND *LACTOBACILLUS RHAMNOSUS*

Because the *Lactobacillus plantarum* strain ATCC8014 used to vaccinate macaques along with iSIV had never been used in humans, we tested the common probiotic strain *Lactobacillus rhamnosus* (LR) as bacterial adjuvant (34). Four macaques were vaccinated orally with iSIV and LR; at the same time, four control macaques received LR only (two animals) and iSIV only (two animals). All macaques were vaccinated according to the protocol used for iSIV + LP vaccination. The *ex vivo* antiviral activity following vaccination increased progressively to reach a ratio >200 in all vaccinated macaques by 2 months post vaccination (**Figure 4A**).

Moreover, all vaccinated animals were sterilely protected from an intrarectal challenge (100,000 $TCID_{50}$ of SIVmac239) performed 12 weeks post vaccination (**Figures 4B,C**).

In summary, in the macaque model, we have demonstrated the prevention of SIV infection by inducing immunological tolerance against the infectious agent. The administration of iSIVmac239 with bacterial adjuvants such as BCG, LP, or LR, stimulated macaques to develop a thus far unrecognized type of SIV-specific

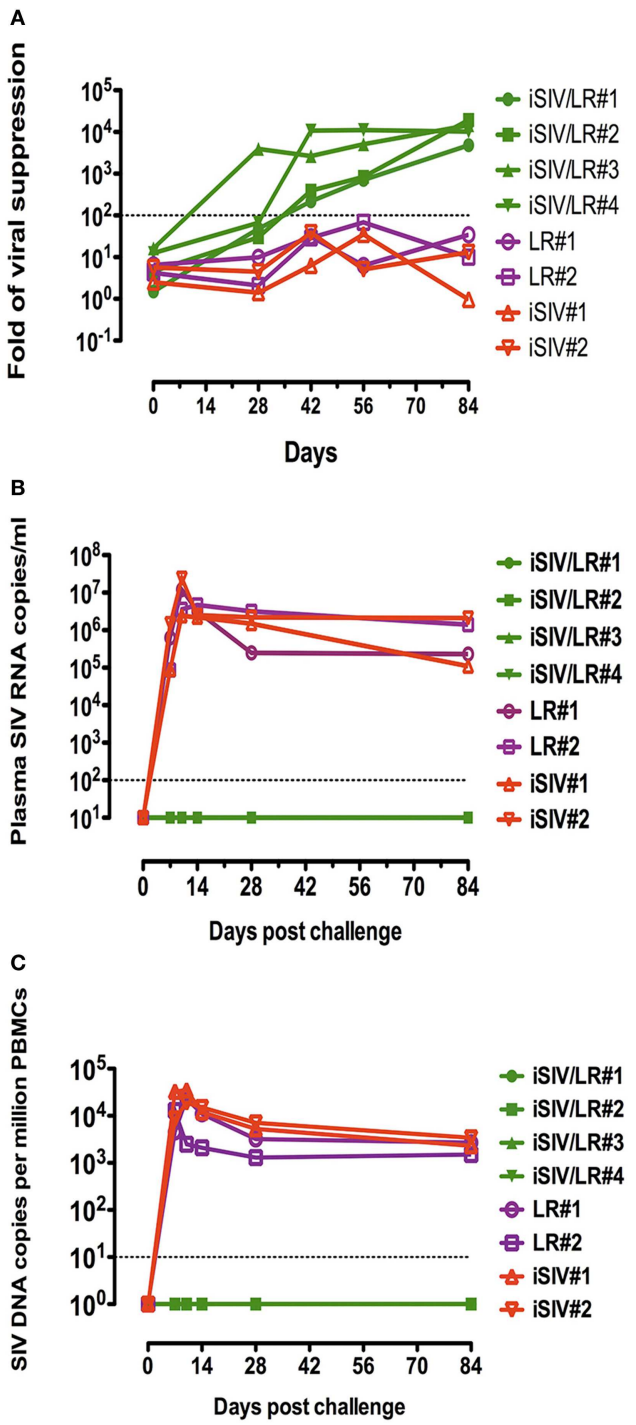


FIGURE 4 | *Ex vivo* antiviral activity induced by CD8$^+$ T-cells of four macaques intragastrically immunized with iSIV plus LR and viral loads of the four macaques following an intrarectal challenge. (A) *Ex vivo* antiviral activity induced by CD8$^+$ T-cells of four macaques intragastrically immunized with iSIV and LR and four control monkeys immunized with iSIV alone or LR alone; **(B,C)** plasma viral loads **(B)** and PBMC proviral loads **(C)** following an intrarectal challenge performed intrarectally with SIVmac239 (100,000 TCID50) at day 90 post-immunization.

immune tolerance characterized by the activation of a previously unrecognized population of non-cytolytic MHCIb/E-restricted CD8$^+$ T-regulatory cells that have the apparent ability to suppress the activation of SIV-positive CD4$^+$ T-cells. This suppression resulted in the blocking of SIV reverse transcription in CD4$^+$ T-cells thereby preventing the initial burst of virus replication and thus protecting macaques from infection. Out of 24 intragastrically vaccinated macaques challenged with a high dose of SIVmac239 or the heterologous strain SIVB670 3 to 14 months later via the intrarectal route, 23 were sterilely protected for up to 48 months while all control macaques became infected.

DISCUSSION

We have identified three areas of research that will be required to establish the mechanisms involved in the observed protection.

CHARACTERISTICS OF VACCINE-INDUCED CD8$^+$ T-REGULATORY CELLS

The suppressive non-cytolytic MHC-IB/E-restricted CD8$^+$ T-cells identified in the present study represent a new class of CD8$^+$ T-regulatory cells (CD8$^+$ Tregs) that has not been described previously in the context of any antiviral vaccination or in SIV or HIV infection. Thus far, the only T-regulatory cells described in HIV infection were classical Tregs (CD25$^+$FoxP3$^+$CD4$^+$ T-cells) known to be involved in some models of immune tolerance (35). Their role in HIV infection is not clearly understood; on the one hand, the expansion of Tregs has been shown to be associated with the suppression of HIV-specific CD4$^+$ T-cell responses and disease progression (36, 37) while on the other hand, Tregs have been associated with protection from productive infection, CD4$^+$ T-cell activation and disease progressions in both humans (38, 39) and non-human primates (40). In the present study, the *ex vivo* removal of CD25$^+$FoxP3$^+$CD4$^+$ T-cells by an anti-CD25 antibody did not modify either the suppression of CD4$^+$ T-cell activation or that of viral replication. Except for the fact that they are non-cytolytic, the CD8$^+$ Tregs observed here resemble those which targeted and eliminated abnormally activated antigen-specific CD4$^+$ T-helper cells in the mouse model (41, 42) where the inhibitory interaction depends on recognition of surface Qa-1, corresponding to MHC-IB/E in macaques and to HLA-E in humans (43), expressed by aberrantly activated target cells (44, 45). Similar CD8$^+$ Tregs have also been implicated in the control of autoimmune type 1 diabetes in humans (46). Interestingly, we also found CD8$^+$ Tregs with the same characteristics in human elite controllers, a small percentage of HIV-infected patients (<1%) who have naturally long-term undetectable viral loads but harbor the virus in their target cells (Wei Lu and Jean-Marie Andrieu, manuscript in preparation). So far, we have not been able to identify the CD antigens specifically associated with these CD8$^+$ Tregs and their phenotypic and molecular characteristics require further studies.

ROLE OF CD8$^+$ T-REGULATORY CELLS

Because vaccinated macaques challenged via the intrarectal route were negative for both SIV RNA in plasma and proviral SIV DNA in PBMC, our results suggest that virus infection was arrested before nuclear integration. In quiescent CD4$^+$ T-cells,

virus penetration is followed within 2 h by the presentation at the plasma membrane of Gag/Pol and other protein epitopes derived from incoming virions (47) while Env and Nef protein presentation requires *de novo* synthesis (48). The subsequent phases of the infectious process including reverse transcription and proviral DNA integration, develop very inefficiently in quiescent $CD4^+$ T-cells but very efficiently in activated $CD4^+$ T-cells (49–51). This is in keeping with the notion that the early activation of a small founder population of infected $CD4^+$ T-cells at the portal of entry is required for the local expansion and establishment of systemic infection (52, 53). This early event potentially gives our vaccine-induced $CD8^+$ T-cells the opportunity to arrest the infectious process before proviral DNA integration. We observed that the withdrawal of our vaccine-induced $CD8^+$ T-cells from PBMC cultures before $CD4^+$ T-cell activation, allowed $CD4^+$ T-cells to become activated and thereby viral replication to proceed. Similarly, *in vivo* depletion of the $CD8^+$ T-cells performed at the time of intrarectal challenge allowed $CD4^+$ T-cells to be activated and the virus to replicate. Such replication, however, was brought under control as the novel $CD8^+$ T-cells recovered in lymphoid organs and the blood stream. We showed in *ex vivo* cultures that the inhibition of viral replication was directly attributable to this new class of $CD8^+$ Tregs. This is also highly likely to be the case *in vivo* although direct evidence for this is lacking since we depleted the whole $CD8^+$ T-cell population rather than specifically the Treg subset. In monkeys intravenously challenged, the transient peak of viral replication followed by the residual presence of cellular SIV DNA suggested that infectious SIV particles that entered the body by this route encountered activated $CD4^+$ T-cells, probably in secondary lymphoid organs, which allowed the virus to complete the first cycles of replication. However, it cannot be excluded that non-activated (quiescent) $CD4^+$ T-cells, which were penetrated by newly released virions, were prevented from becoming activated by the vaccine-generated $CD8^+$ Treg. An in-depth understanding of the results presented here will require extensive exploratory studies of cellular and molecular immunovirology.

INDUCTION OF SIV-SPECIFIC $CD8^+$ T-REGULATORY CELLS

This remains the most mysterious unanswered question. When iSIV, a particulate immunogen was administered alone via the intragastric route, it stimulated the intestinal immune system to induce the activation and proliferation of SIV-$CD4^+$ T-cell as well as the generation of SIV-specific antibodies and interferon γ-producing cells according to the classical vaccine paradigm. However, this iSIV-only vaccination did not induce any *in vitro* or *in vivo* viral suppression nor any protection in challenged macaques. On the other hand, a soluble protein such as SIV P55, when intragastrically administered in its own, did not induce any immunological response nor any challenge protection (data not shown). In surprising contrast, when iSIV was administered along with BCG, or LP or LR, the reaction of the macaque intestinal immune was totally different. SIV-$CD4^+$ T-cells proliferation and activation were abolished (through the contact between the TCRαβ of the vaccine-induced $CD8^+$ T-regulatory cells and the MHCIb/E of SIV positive $CD4^+$ T-cells). A first consequence of this vaccine-induced SIV-positive $CD4^+$ T-cell unresponsiveness/anergy is most likely the suppression of any help to the induction of SIV-specific antibody- and interferon γ-producing cells; the observation of such an antigen-specific immune tolerance opens a vast array of research on the suppressive arm of the immune system and its potential manipulation in human and veterinary medicine. The other consequence of this vaccine-induced SIV-positive $CD4^+$ T-cell unresponsiveness/anergy is of unique importance for vaccinologists since it is responsible for the suppression of *in vitro* and *in vivo* SIV reverse transcription and for the sterile protection of macaques against SIV challenge, bringing the experimental confirmation of our initial suspicion that suppression of $CD4^+$ T-cell activation is able to suppress viral replication (9).

The mechanism of this extraordinary immunological switch is so far unknown. BCG itself has never been suspected to possess tolerogenic properties. Only in Brazil, have infants been given BCG via the oral route (over a period of 50 years from the 20s to the 70s) and Brazilian scientists found that oral BCG protected against childhood tuberculosis, meningitis, and miliary disease with the same efficacy as intradermic BCG (54). However, orally vaccinated infants remained generally unresponsive/anergic to the post vaccination tuberculin test, which became generally positive after classical intradermic vaccination (55). In the same line, oral BCG vaccination inhibited delayed-type hypersensitivity to purified protein derivative (while at the same time it induced interferon γ-secreting T-cells upon *Mycobacterium* antigens stimulation) (56). Moreover, it has also been observed that when the central nervous system of mice was infected with BCG, the mice were able to overcome experimental autoimmune encephalomyelitis, a classical mice model of autoimmune disease (57). The possibility that BCG may possess certain unexplored tolerogenic properties seems worthy of further research inasmuch as $CD8^+$ Tregs have been very recently shown to dominate suppressive phenotype and function after BCG activation of human cells (58).

On the other hand, there is some experimental evidence suggesting that LP and LR may have some tolerogenic properties (30–32), which may be involved in their probiotic ability to fight human inflammatory bowel disease (59). However, the mechanism of induction of such a tolerance seems to be via the generation of classical $CD25^+FoxP3^+CD4^+$ Tregs (60, 61) and not through the intervention of $CD8^+$ Tregs.

It must be mentioned that recombinant BCG expressing viral antigens including HIV and SIV proteins has been tested via the mucosal route in non-human primates. These recombinant BCGs induced IgA against BCG-expressed viral proteins but were never found to induce any humoral or cellular sign of virus-induced immune tolerance (62). Similarly, lactobacilli and lactococci engineered to express antigenic proteins also including SIV antigens have been mucosally administered to different animal models including non-human primates; whatever the antigen expressed, the immunological result of the mucosal administration was always the production of specific of antigenic protein-directed antibodies and particularly of IgA by the intestinal immune system (63).

Our results demonstrate that an oral vaccine comprising iSIV, a particulate immunogen adjuvanted by BCG, LP, or LR administered intragastrically to a large number of macaques stimulated their intestinal immune system and generated a new class of $CD8^+$ Tregs that suppressed the activation of mucosal and systemic SIV-positive $CD4^+$ T-cells. This suppression of $CD4^+$

T-cell activation inhibited viral replication and thereby prevented SIV infection in the macaque model. The protective effect of this innovative immunization regimen against SIV challenge, together with the identification of a correlate of protection *ex vivo*, is quite striking. Given that SIV and HIV require activated $CD4^+$ T-cells in which to replicate, this tolerogenic vaccine approach may offer an exciting new avenue in preventive HIV vaccine research. We are preparing a randomized phase 1 trial where a small population of non-at-risk volunteers will receive the vaccine or a placebo; the vaccination success will be defined by the increase of the *ex vivo* antiviral activity of $CD8^+$ T-cells (ratio >200) in the vaccinated group while it will remain low and stable (ratio <100) in the placebo group. On the other hand, the *de novo* induction of this class of $CD8^+$ Tregs could potentially be used therapeutically to maintain HIV replication suppression in infected patients in whom antiviral treatments have been interrupted. We are now preparing a phase 1 trial where infected volunteers with undetectable viral loads under antiviral therapy will be orally vaccinated with heat-inactivated HIV and LR; the antiviral treatment of infected volunteers will be suspended at week 24. The success of this trial will be ascertained by the increase of *ex vivo* antiviral activity of $CD8^+$ T-cells (ratio >200) by 8 weeks post vaccination and by permanent undetectable plasma viral loads from week 24 (antiviral treatment withdrawal). The new tolerogenic vaccine paradigm described in the present study, beside its potential use in the HIV vaccine field, could potentially be exploited in the management of a wide range of immune disorders and could uncover so far unrecognized immunological mechanisms.

ACKNOWLEDGMENTS

This work was funded by the Institut de Recherches sur les Vaccins et l'Immunothérapie des Cancers et du SIDA, Paris, France and Biovaxim Ltd., London, UK. We thank W. Deng, V. Fabre-Jagot, L. Yu, J. Yuan, and Y. Zhou for technical assistance. We are grateful to Marc Van Regenmortel for helpful discussions during the preparation of the manuscript and Jeffrey Almond for his critical review of the manuscript. We would also like to thank very much Sven Britton, Philippe Even, Stefano Marullo, Hans Wigzell, and Jean-Claude Weill for fruitful discussions and support.

REFERENCES

1. Plotkin SA. Correlates of protection induced by vaccination. *Clin Vaccine Immunol* (2010) **7**:1055–65. doi:10.1128/CVI.00131-10
2. Barré-Sinoussi F, Chermann JC, Rey F, Nugeyre MT, Chamaret S, Gruest J, et al. Isolation of a T-lymphotropic retrovirus from a patient at risk for acquired immune deficiency syndrome (AIDS). *Science* (1983) **220**(4599):868–71. doi:10.1126/science.6189183
3. Esparza J. A brief history of the global effort to develop a preventive HIV vaccine. *Vaccine* (2013) **31**(35):3502–18. doi:10.1016/j.vaccine.2013.05.018
4. Rerks-Ngarm S, Pitisuttithum P, Nitayaphan S, Kaewkungwal J, Chiu J, Paris R, et al. Vaccination with ALVAC and AIDSVAX to prevent HIV-1 infection in Thailand. *N Engl J Med* (2009) **361**:2209–20. doi:10.1056/NEJMoa0908492
5. Duerr A, Huang Y, Buchbinder S, Coombs RW, Sanchez J, del Rio C, et al. Extended follow-up confirms early vaccine-enhanced risk of HIV acquisition and demonstrates waning effect over time among participants in a randomized trial of recombinant adenovirus HIV vaccine (Step Study). *J Infect Dis* (2012) **206**(2):258–66. doi:10.1093/infdis/jis342
6. Gray GE, Moodie Z, Metch B, Gilbert PB, Bekker LG, Churchyard G, et al. Recombinant adenovirus type 5 HIV gag/pol/nef vaccine in South Africa: unblinded, long-term follow-up of the phase 2b HVTN 503/Phambili study. *Lancet Infect Dis* (2014) **14**(5):388–96. doi:10.1016/S1473-3099(14)70020-9
7. Hammer SM, Sobieszczyk ME, Janes H, Karuna ST, Mulligan MJ, Grove D, et al. Efficacy trial of a DNA/rAd5 HIV-1 preventive vaccine. *N Engl J Med* (2013) **369**(22):2083–92. doi:10.1056/NEJMoa1310566
8. Fauci AS, Marovich MA, Dieffenbach CW, Hunter E, Buchbinder SP. Immunology. Immune activation with HIV vaccines. *Science* (2014) **344**(6179):49–51. doi:10.1126/science.1250672
9. Andrieu JM, Even P, Venet A. AIDS and related syndromes as a viral-induced autoimmune disease of the immune system: an anti-MHC II disorder. Therapeutic implications. *AIDS Res* (1986) **2**(3):163–74. doi:10.1089/aid.1.1986.2.163
10. Paiardini M, Müller-Trutwin M. HIV-associated chronic immune activation. *Immunol Rev* (2013) **254**(1):78–101. doi:10.1111/imr.12079
11. Klatt NR, Chomont N, Douek DC, Deeks SG. Immune activation and HIV persistence: implications for curative approaches to HIV infection. *Immunol Rev* (2013) **254**(1):326–42. doi:10.1111/imr.12065
12. Andrieu JM, Even P, Venet A, Tourani JM, Stern M, Lowenstein W, et al. Effects of cyclosporine on T-cell subsets in human immunodeficiency virus disease. *Clin Immunol Immunopathol* (1988) **46**:181–98. doi:10.1016/0090-1229(88)90071-2
13. Levy R, Jais JP, Tourani JM, Even P, Andrieu JM. Long-term follow-up of HIV positive asymptomatic patients having received cyclosporine A. *Adv Exp Med Biol* (1995) **374**:229–34. doi:10.1007/978-1-4615-1995-9_20
14. Andrieu JM, Lu W, Levy R. Sustained increases in CD4 cell counts in asymptomatic human immunodeficiency virus type 1-seropositive patients treated with prednisolone for 1 year. *J Infect Dis* (1995) **171**(3):523–30. doi:10.1093/infdis/171.3.523
15. Andrieu JM, Lu W. Long-term clinical, immunologic and virologic impact of glucocorticoids on the chronic phase of HIV infection. *BMC Med* (2004) **2**:17. doi:10.1186/1741-7015-2-17
16. Lu W, Salerno-Goncalves R, Yuan J, Sylvie D, Han DS, Andrieu JM. Glucocorticoids rescue CD4+ T-lymphocytes from activation-induced apoptosis triggered by HIV-1: implications for pathogenesis & therapy. *AIDS* (1995) **9**:35–42. doi:10.1097/00002030-199501000-00005
17. Lu W, Andrieu JM. In vitro human immunodeficiency virus eradication by autologous CD8(+) T-cells expanded with inactivated-virus-pulsed dendritic cells. *J Virol* (2001) **75**(19):8949–56. doi:10.1128/JVI.75.19.8949-8956.2001
18. Lu W, Wu X, Lu Y, Guo W, Andrieu JM. Therapeutic dendritic-cell vaccine for simian AIDS. *Nat Med* (2003) **9**:27–32. doi:10.1038/nm806
19. Lu W, Arraes LC, Ferreira WT, Andrieu JM. Therapeutic dendritic-cell vaccine for chronic HIV-1 infection. *Nat Med* (2004) **10**:1359–65. doi:10.1038/nm1147
20. García F, Climent N, Guardo AC, Gil C, León A, Autran B, et al. A dendritic cell-based vaccine elicits T cell responses associated with control of HIV-1 replication. *Sci Transl Med* (2013) **5**(166):166ra2. doi:10.1126/scitranslmed.3004682
21. Chen S, Lai C, Wu X, Lu Y, Han D, Guo W, et al. Variability of bio-clinical parameters in Chinese-origin Rhesus macaques infected with simian immunodeficiency virus: a nonhuman primate AIDS model. *PLoS One* (2011) **6**:e23177. doi:10.1371/journal.pone.0023177
22. Stahl-Hennig C, Suh YS, Park KS, Sauermann U, Kim KS, Ahn S, et al. Immunogenicity of a DNA prime and recombinant adenovirus boost regime significantly varies between rhesus macaques of Chinese and Indian origins. *J Med Primatol* (2007) **36**:195–205. doi:10.1111/j.1600-0684.2007.00237.x
23. Lu W, Chen S, Lai C, Guo W, Fu L, Andrieu JM. Induction of CD8+ regulatory T-cells protects macaques against SIV challenge. *Cell Rep* (2012) **2**(6):1736–46. doi:10.1016/j.celrep.2012.11.016
24. Mangtani P, Abubakar I, Ariti C, Beynon R, Pimpin L, Fine PE, et al. Protection by BCG vaccine against tuberculosis: a systematic review of randomized controlled trials. *Clin Infect Dis* (2014) **58**(4):470–80. doi:10.1093/cid/cit790
25. Ramoner R, Rieser C, Herold M, Klocker H, Bartsch G, Stenzl A, et al. Activation of human dendritic cells by bacillus Calmette-Guerin. *J Urol* (1998) **159**(5):1488–92. doi:10.1097/00005392-199805000-00021
26. Demangel C, Britton WJ. Interaction of dendritic cells with mycobacteria: where the action starts. *Immunol Cell Biol* (2000) **78**:318–24. doi:10.1046/j.1440-1711.2000.00935.x
27. Gandhi NM, Morales A, Lamm DL. Bacillus Calmette-Guérin immunotherapy for genitourinary cancer. *BJU Int* (2013) **112**(3):288–97. doi:10.1111/j.1464-410X.2012.11754.x
28. Stewart JH IV, Levine EA. Role of bacillus Calmette-Guérin in the treatment of advanced melanoma. *Expert Rev Anticancer Ther* (2011) **11**(11):1671–6. doi:10.1586/era.11.163
29. Benévolo-de-Andrade TC, Monteiro-Maia R, Cosgrove C, Castello-Branco LR. BCG Moreau Rio de Janeiro – an oral vaccine against tuberculosis – review.

Mem Inst Oswaldo Cruz (2005) **100**(5):459–65. doi:10.1590/S0074-02762005000500002
30. Grangette C, Nutten S, Palumbo E, Morath S, Hermann C, Dewulf J. Enhanced anti-inflammatory capacity of a *Lactobacillus plantarum* mutant synthesizing modified teichoic acids. *Proc Natl Acad Sci USA* (2005) **102**:10321–6. doi:10.1073/pnas.0504084102
31. van Baarlen P, Troost FJ, van Hemert S, van der Meer C, de Vos WM, de Groot PJ, et al. Differential NF-kappa B pathways induction by *Lactobacillus plantarum* in the duodenum of healthy humans correlating with immune tolerance. *Proc Natl Acad Sci U S A* (2009) **106**:2371–6. doi:10.1073/pnas.0809919106
32. Baba N, Samson S, Bourdet-Sicard R, Rubio M, Sarfati M. Commensal bacteria trigger a full dendritic cell maturation program that promotes the expansion of non-Tr1 suppressor T-cells. *J Leukoc Biol* (2008) **84**:1468–76. doi:10.1189/jlb.0108017
33. Kleerebezem M, Boekhorst J, van Kranenburg R, Molenaar D, Kuipers OP, Leer R. Complete genome sequence of *Lactobacillus plantarum* WCFS1. *Proc Natl Acad Sci USA* (2003) **100**:1990–5. doi:10.1073/pnas.0337704100
34. Nivoliez A, Camares O, Paquet-Gachinat M, Bornes S, Forestier C, Veisseire P. Influence of manufacturing processes on in vitro properties of the probiotic strain *Lactobacillus rhamnosus* Lcr35®. *J Biotechnol* (2012) **160**(3–4):236–41. doi:10.1016/j.jbiotec.2012.04.005
35. Faria AM, Weiner HL. Oral tolerance. *Immunol Rev* (2005) **206**:232–59.
36. Aandahl EM, Michaelsson J, Moretto WJ, Hecht FM, Nixon DF. Human CD4+ CD25+ regulatory T-cells control T-cell responses to human immunodeficiency virus and cytomegalovirus antigens. *J Virol* (2004) **78**:2454–9. doi:10.1128/JVI.78.5.2454-2459.2004
37. Nilsson J, Boasso A, Velilla PA, Zhang R, Vaccari M, Franchini G, et al. HIV-1-driven regulatory T-cell accumulation in lymphoid tissues is associated with disease progression in HIV/AIDS. *Blood* (2006) **108**:3808–17. doi:10.1182/blood-2006-05-021576
38. Card CM, McLaren PJ, Wachihi C, Kimani J, Plummer FA, Fowke KR. Decreased immune activation in resistance to HIV-1 infection is associated with an elevated frequency of CD4(+)CD25(+)FOXP3(+) regulatory T-cells. *J Infect Dis* (2009) **199**:1318–22. doi:10.1086/597801
39. Chase AJ, Yang HC, Zhang H, Blankson JN, Siliciano RF. Preservation of FoxP3+ regulatory T-cells in the peripheral blood of human immunodeficiency virus type 1-infected elite suppressors correlates with low CD4+ T-cell activation. *J Virol* (2008) **82**:8307–15. doi:10.1128/JVI.00520-08
40. Kornfeld C, Ploquin MJ, Pandrea I, Faye A, Onanga R, Apetrei C, et al. Antiinflammatory profiles during primary SIV infection in African green monkeys are associated with protection against AIDS. *J Clin Invest* (2005) **115**:1082–91. doi:10.1172/JCI200523006
41. Kim HJ, Verbinnen B, Tang X, Lu L, Cantor H. Inhibition of follicular T-helper cells by CD8(+) regulatory T-cells is essential for self tolerance. *Nature* (2010) **467**:328–32. doi:10.1038/nature09370
42. Sarantopoulos S, Lu L, Cantor H. Qa-1 restriction of CD8+ suppressor T-cells. *J Clin Invest* (2004) **114**:1218–21. doi:10.1172/JCI200423152
43. Pietra G, Romagnani C, Manzini C, Moretta L, Mingari MC. The emerging role of HLA-E-restricted CD8+ T-lymphocytes in the adaptive immune response to pathogens and tumors. *J Biomed Biotechnol* (2010) **2010**:907–14. doi:10.1155/2010/907092
44. Kim HJ, Wang X, Radfar S, Sproule TJ, Roopenian DC, Cantor H. CD8+ T regulatory cells express the Ly49 Class I MHC receptor and are defective in autoimmune prone B6-Yaa mice. *Proc Natl Acad Sci U S A* (2011) **108**:2010–5. doi:10.1073/pnas.1018974108
45. Nagarajan NA, Gonzalez F, Shastri N. Nonclassical MHC class Ib-restricted cytotoxic T-cells monitor antigen processing in the endoplasmic reticulum. *Nat Immunol* (2012) **13**:579–86. doi:10.1038/ni.2282
46. Jiang H, Canfield SM, Gallagher M, Jiang HH, Jiang Y, Zheng Z, et al. HLA-E-restricted regulatory CD8(+) T-cells are involved in development and control of human autoimmune type 1 diabetes. *J Clin Invest* (2010) **120**:3641–50. doi:10.1172/JCI43522
47. Sacha JB, Chung C, Rakasz EG, Spencer SP, Jonas AK, Bean AT. Gag-specific CD8+ T-lymphocytes recognize infected cells before AIDS-virus integration and viral protein expression. *J Immunol* (2007) **178**:2746–54. doi:10.4049/jimmunol.178.5.2746
48. Sacha JB, Chung C, Reed J, Jonas AK, Bean AT, Spencer SP, et al. Pol-specific CD8+ T-cells recognize simian immunodeficiency virus-infected cells prior to Nef-mediated major histocompatibility complex class I down regulation. *J Virol* (2007) **81**:11703–12. doi:10.1128/JVI.00926-07
49. Korin YD, Zack JA. Nonproductive human immunodeficiency virus type 1 infection in nucleoside-treated G0 lymphocytes. *J Virol* (1999) **73**:6526–32.
50. Zack JA, Arrigo S, Weitsman SR, Go AS, Haislip A, Chen IS. HIV-1 entry into quiescent primary lymphocytes: molecular analysis reveals a labile, latent viral structure. *Cell* (1990) **61**:213–22.
51. Vatakis DN, Kim S, Kim N, Chow SA, Zack JA. Human immunodeficiency virus integration efficiency and site selection in quiescent CD4+ T-cells. *J Virol* (2009) **83**:6222–33. doi:10.1128/JVI.00356-09
52. Haase AT. Early events in sexual transmission of HIV and SIV and opportunities for interventions. *Annu Rev Med* (2012) **62**:127–39. doi:10.1146/annurev-med-080709-124959
53. Zhang Z, Wietgrefe SW, Li Q, Shore MD, Duan L, Reilly C, et al. Roles of substrate availability and infection of resting and activated CD4+ T-cells in transmission and acute simian immunodeficiency virus infection. *Proc Natl Acad Sci U S A* (2004) **101**:5640–5. doi:10.1073/pnas.0308425101
54. Antas PR, Castello-Branco LR. New vaccines against tuberculosis: lessons learned from BCG immunization in Brazil. *Trans R Soc Trop Med Hyg* (2008) **102**(7):628–30. doi:10.1016/j.trstmh.2008.03.014
55. Menzies D. What does tuberculin reactivity after bacille Calmette-Guérin vaccination tell us? *Clin Infect Dis* (2000) **31**(Suppl 3):S71–4. doi:10.1086/314075
56. Hoft DF, Brown RM, Belshe RB. Mucosal bacille Calmette-Guérin vaccination of humans inhibits delayed-type hypersensitivity to purified protein derivative but induces mycobacteria-specific interferon-gamma responses. *Clin Infect Dis* (2000) **30**(Suppl 3):S217–22. doi:10.1086/313864
57. Lee J, Reinke EK, Zozulya AL, Sandor M, Fabry Z. *Mycobacterium bovis* bacille Calmette-Guérin infection in the CNS suppresses experimental autoimmune encephalomyelitis and Th17 responses in an IFN-gamma-independent manner. *J Immunol* (2008) **181**(9):6201–12. doi:10.4049/jimmunol.181.9.6201
58. Boer MC, van Meijgaarden KE, Joosten SA, Ottenhoff TH. CD8+ regulatory T-cells, and not CD4+ T-cells, dominate suppressive phenotype and function after in vitro live *Mycobacterium bovis*-BCG activation of human cells. *PLoS One* (2014) **9**(4):e94192. doi:10.1371/journal.pone.0094192
59. Sheil B, Shanahan F, O'Mahony L. Probiotic effects on inflammatory bowel disease. *J Nutr* (2007) **137**(3 Suppl 2):819S–24S.
60. Smits HH, Engering A, van der Kleij D, de Jong EC, Schipper K, van Capel TM, et al. Selective probiotic bacteria induce IL-10-producing regulatory T-cells in vitro by modulating dendritic cell function through dendritic cell-specific intercellular adhesion molecule 3-grabbing nonintegrin. *J Allergy Clin Immunol* (2005) **115**(6):1260–7. doi:10.1016/j.jaci.2005.03.036
61. Scott CL, Aumeunier AM, Mowat AM. Intestinal CD103+ dendritic cells: master regulators of tolerance? *Trends Immunol* (2011) **32**(9):412–9. doi:10.1016/j.it.2011.06.003
62. Chapman R, Chege G, Shephard E, Stutz H, Williamson AL. Recombinant *Mycobacterium bovis* BCG as an HIV vaccine vector. Review. *Curr HIV Res* (2010) **8**(4):282–98. doi:10.2174/157016210791208686
63. Xin KQ, Hoshino Y, Toda Y, Igimi S, Kojima Y, Jounai N, et al. Immunogenicity and protective efficacy of orally administered recombinant *Lactococcus lactis* expressing surface-bound HIV Env. *Blood* (2003) **102**(1):223–8. doi:10.1182/blood-2003-01-0110

Conflict of Interest Statement: Jean-Marie Andrieu and Wei Lu have received grants from and are shareholders of Biovaxim Ltd. The other co-authors report no conflicts of interest.

Received: 29 May 2014; accepted: 11 June 2014; published online: 30 June 2014.
*Citation: Andrieu J-M, Chen S, Lai C, Guo W and Lu W (2014) Mucosal SIV vaccines comprising inactivated virus particles and bacterial adjuvants induce CD8$^+$ T-regulatory cells that suppress SIV-positive CD4$^+$ T-cell activation and prevent SIV infection in the macaque model. Front. Immunol. **5**:297. doi: 10.3389/fimmu.2014.00297*
This article was submitted to HIV and AIDS, a section of the journal Frontiers in Immunology.

frontiers in
IMMUNOLOGY

GENERAL COMMENTARY
published: 14 July 2014
doi: 10.3389/fimmu.2014.00329

More surprises in the development of an HIV vaccine

José Esparza*[1] *and Marc H. V. Van Regenmortel*[2]

[1] *Institute of Human Virology, University of Maryland School of Medicine, Baltimore, MD, USA*
[2] *CNRS, UMR7242-Institut de Recherche de l'Ecole de Biotechnologie de Strasbourg (IREBS), Université de Strasbourg, Illkirch-Graffenstaden, France*
**Correspondence: vanregen@unistra.fr*

Edited by:
Francesca Chiodi, Karolinska Institutet, Sweden

Reviewed by:
Francesca Chiodi, Karolinska Institutet, Sweden
Bence Rethi, Karolinska Institutet, Sweden

Keywords: inactivated SIV, bacterial adjuvants, lactobacilli, SIV vaccine, HIV vaccine, tolerogenic vaccine

A commentary on

Mucosal SIV vaccines comprising inactivated virus particles and bacterial adjuvants induce CD8+ T-regulatory cells that suppress SIV positive CD4+ T-cell activation and prevent SIV infection in the macaque model
*by Andrieu JM, Chen S, Lai C, Guo W, Lu W. Front Immunol (2014) **5**:297. doi: 10.3389/fimmu.2014.00297*

In the current issue of Frontiers in Immunology, Jean-Marie Andrieu and collaborators, report results from non-human primate experiments designed to explore a new vaccine concept aimed at inducing tolerance to the simian immunodeficiency virus (SIV) (1). This approach, which is significantly different from other vaccine concepts tested to date, resulted in a surprisingly high level of protection. If the results are confirmed and extended to the human immunodeficiency virus (HIV), this approach may represent a game changing strategy, which should be welcomed by a field that has been marred by mostly disappointing results.

When HIV was discovered and established as the cause of the Acquired Immune Deficiency Syndrome (AIDS) in 1983–1984, there was an expectation that a preventive vaccine would be rapidly developed (2).

Vaccines against several major human viral diseases (polio, measles, mumps, rubella, etc.) were successfully developed during the preceding two or three decades, mostly using live-attenuated viruses, and designed to induce the same type of protective immune responses that develop after natural infection. Moreover, recent advances in molecular biology and recombinant DNA technologies were offering exciting new opportunities for vaccine development, first achieved with the licensure in 1986 of a recombinant vaccine against hepatitis B (3, 4).

Since the use of whole-inactivated or of live-attenuated vaccines was considered too risky for a pathogen such as HIV, the molecular approach was the one selected by early HIV vaccine developers. That decision was also based on the confidence that new knowledge on the structure and function of the virus, as well as of the pathogenesis of the disease, will provide the information needed for the rational development of a much needed HIV vaccine (5).

In that environment of optimism, the first phase I clinical trials of HIV vaccines started in the United States in 1988. Since then, more than 200 clinical trials have been conducted globally, the majority of them phase I and II trials, to assess the safety and immunogenicity of different vaccine candidates. Those candidate vaccines were developed and tested according to prevailing paradigms that sequentially explored the role of neutralizing antibodies, cell-mediated immunity (CMI) and, more recently, other potential mechanisms of immune protection (2, 6).

Although much has been learned from those small-scale clinical trials, the results from phase IIb/III efficacy trials are the ones that have driven major changes on how HIV vaccine research is advanced. Those trials have also given us a few surprises. Fortunately, the field has been able to learn from those lessons and steadily move forward.

Perhaps the first major surprise was when in 1994 we learned that field isolates of HIV were more difficult to neutralize *in vitro* than laboratory-adapted strains, and that proposed existing candidate vaccines could not induce the appropriate type of neutralizing antibodies, a problem that we are still struggling to solve. Nevertheless, in the early 2000s, two gp120 candidate vaccines from VaxGen were tested in efficacy trials and, as many predicted, they failed to protect. That failure shifted the field to CMI vaccines and to the suggestion that perhaps the best that an HIV vaccine could do is to decrease virus load in vaccinated individuals who became infected (7). Unfortunately, the STEP study, which tested the CMI concept using an adenovirus 5 vector, and which was a favorite approach of the HIV vaccine community, was halted in 2007 because of lack of efficacy (8). That was a major surprise that led to calls to slow down clinical trials and to go back to basic science (9).

The next major surprise came in 2009, when the results from the Thai RV144 were announced. The trial, which evaluated a canarypox prime followed by a gp120 boost, was strongly opposed by some of the leading HIV vaccine scientists (10). Unexpectedly, the trial showed for the first time that prevention of HIV infection was achievable by an HIV vaccine (11). In a commentary authored by the late Norman Letvin (12), who himself expressed concerns about the conduct of the RV144 trial, he indicated that the findings were not expected based on preclinical studies and human immunogenicity data, concluding with the lapidary remark that "*we have learned to expect the unexpected in our efforts to generate an effective HIV vaccine.*"

Although the observed protection in RV144 was modest (31.2%), those results

not only brought new optimism to the field, but also triggered a major collaborative effort to try to identify immune correlates of protection (13). In this regard, novel and more promising vaccines are being developed that may result in higher levels of protective efficacy, including the use of vectors based on adenovirus 26 (Ad26) and cytomegalovirus (CMV) (14, 15).

Another surprise came when a careful statistical analysis of the step study confirmed that vaccination in fact enhanced HIV acquisition among a subset of the volunteers (16), an observation that was also been made in the Phambili study conducted in South Africa using the same vaccine as in the step study (17). The most likely explanation of the observed enhancement is a specific immune activation induced by the adenovirus 5 vectored vaccines. Although the mechanism is poorly understood, it does not seem to be present with another adenovirus 5 vectored HIV vaccine (18), and it is not clear how relevant it could be to other vaccine approaches (19). Nevertheless, it is well-known that activation of $CD4^+$ cells is important for HIV replication, which creates a dilemma for vaccinologists, who have to thread a compromise between the desire to induce strong vaccine responses and, at the same time, avoid the immune activation that may enhanced HIV acquisition. In this and other regards, HIV/AIDS is different from other viral diseases for which vaccines have been developed, because forces vaccine developers to explore mechanisms that nature has not developed, especially when dealing with chronic infections (20).

It is in this context that Jean-Marie Andrieu and collaborators report in this journal (1) additional results from an approach that they first reported in 2012 (21, 22).

The investigators used Chinese macaques to explore the concept that the induction of immune tolerance to SIV induces protective immunity in the absence of usual humoral or cellular immune responses. To achieve that goal, inactivated SIV was intragastrically administered together with living bacterial adjuvants (BCG, *Lactobacillus plantarum*, or *Lactobacillus rhamnosus*) with the goal of inducing tolerance to the SIV antigens. In a series of experiments, the investigators showed that their approach protected the experimental animals from mucosal and parenteral challenges. Vaccination neither elicit SIV-specific antibodies nor cytotoxic T-lymphocytes but induced a previously unrecognized population of non-cytolytic MHCIb/E-restricted $CD8^+$ T regulatory cells that suppressed the activation of SIV positive $CD4^+$ T-lymphocytes. Although the number of monkeys is relatively small, the levels of protection are impressive, with 23 out of 24 animals protected in one of the experiments, when animals were challenged 48 months after vaccination.

The 2012 publication from this group (21) had very little impact in the field, perhaps because it was received with a degree of skepticism. After all, 30 years of intense vaccine research had not resulted in a practical effective vaccine, although an HIV vaccine is sorely needed to bring the HIV epidemic under control. No stone should remain unturned in its search, and the approach reported in this journal should not be dismissed *a priori*. Instead, it should be carefully considered by other scientists and appropriately confirmed or refuted by additional research.

In order to accelerate the development of an HIV vaccine, one of us has proposed a number of actions, including the suggestion to establish a program of truly innovative research with protected funding to explore out-of-the-paradigm approaches, perhaps allocating to this program not <10% of the total HIV vaccine investment (23). Out-of-the-paradigm approaches, such as the one proposed by Andrieu et al., should be further explored (24).

Paraphrasing Dean K. Simonton (25), the University of California psychologist who has dedicated his professional life to the study of creativity: good science contributes ideas that are original and useful, and we have plenty of those in the HIV vaccine field. However, the solution to the HIV vaccine challenge will require genius which, according to Simonton, is characterized not only by originality and usefulness, but also by surprising results.

REFERENCES

1. Andrieu JM, Chen S, Lai C, Guo W, Lu W. Mucosal SIV vaccines comprising inactivated virus particles and bacterial adjuvants induce CD8+T-regulatory cells that suppress SIV positive CD4+cell activation and prevent SIV infection in the macaque model. *Front Immunol* (2014) **5**:297. doi:10.3389/fimmu.2014.00297
2. Esparza J. A brief history of the global effort to develop an HIV vaccine. *Vaccine* (2013) **31**:3502–18. doi:10.1016/j.vaccine.2013.05.018
3. Valenzuela P, Medina A, Rutter WJ, Ammerer G, Hall BD. Synthesis and assembly of hepatitis B virus surface antigen particles in yeast. *Nature* (1982) **298**:347–50. doi:10.1038/298347a0
4. AcAleer WJ, Bynak EB, Maigeter RZ, Wampler DE, Miller WJ, Hilleman MR. Human hepatitis B vaccine from recombinant yeast. *Nature* (1984) **307**:178–80. doi:10.1038/307178a0
5. Hilleman MR. A simplified vaccinologists' vaccinology and the pursuit of a vaccine against AIDS. *Vaccine* (1998) **16**:778–93. doi:10.1016/S0264-410X(97)00272-7
6. Excler JL, Ake J, Robb ML, Kim JH, Plotkin SA. Nonneutralizing functional antibodies: a new 'old' paradigm for HIV vaccines. *Clin Vaccine Immunol* (2014). doi:10.1128/CVI.00230-14
7. Johnston MI, Fauci AS. An HIV vaccine – evolving concepts. *N Engl J Med* (2007) **356**:2073–81. doi:10.1056/NEJMra066267
8. Buchbinder SP, Mehrotra DV, Duerr A, Fitzgerald DW, Mogg R, Li D, et al. Efficacy assessment of a cell-mediated immunity HIV-1 vaccine (the step study): a double-blind, randomized, placebo-controlled, test-of-concept trial. *Lancet* (2008) **372**:1881–93. doi:10.1016/S0140-6736(08)61591-3
9. Kaiser J. Review of vaccine failure prompts to basic science. *Science* (2008) **320**:30–1. doi:10.1126/science.320.5872.30
10. Burton DR, Desrosiers RC, Doms RW, Feinberg MB, Gallo RC, Hahn B, et al. A sound rationale needed for phase III HIV-1 vaccine trials. *Science* (2004) **303**:316. doi:10.1126/science.1094620
11. Rerks-Ngarm S, Pitisuttithum P, Nitayaphan S, Kaewkungwal J, Chiu J, Paris R, et al. Vaccination with ALVAC and AIDSVAX to prevent HIV-1 infection in Thailand. *N Engl J Med* (2009) **361**:2209–20. doi:10.1056/NEJMoa0908492
12. Letvin NL. Moving forward in HIV vaccine development. *Science* (2009) **326**:1196–8. doi:10.1126/science.1183278
13. Haynes BF, Gilbert PB, McElrath MJ, Zolla-Pazner S, Tomaras G, Alam S, et al. Immune-correlates analysis of an HIV-1 vaccine efficacy trial. *N Engl J Med* (2012) **366**:1275–86. doi:10.1056/NEJMoa1113425
14. Baden LR, Walsh SR, Seaman MS, Tucker RP, Krause KH, Patel A, et al. First-in-human evaluation of the safety and immunogenicity of a recombinant adenovirus serotype 26 HIV-1 Env vaccine (IPCAVD 001). *J Infect Dis* (2013) **207**:240–7. doi:10.1093/infdis/jis670
15. Hansen SG, Sacha JB, Hughes CM, Ford JC, Burwitz BJ, Scholz I, et al. Cytomegalovirus vectors violate CD8+ T cell epitope recognition paradigms. *Science* (2013) **340**(6135):1237874. doi:10.1126/science.1237874
16. Duerr A, Huang Y, Buchbinder S, Coombs RW, Sanchez J, del Rio C, et al. Extended follow-up confirms early vaccine-enhanced risk of HIV acquisition and demonstrates waning effect over time among participants in a randomized trial of recombinant adenovirus HIV vaccine (step study).

J Infect Dis (2012) **206**:258–66. doi:10.1093/infdis/jis342
17. Gray GE, Moodie Z, Metch B, Gilbert PB, Bekker L-G, Churchyard G, et al. Recombinant adenovirus type 5 HIV gag/pol/nef vaccine in South Africa: unblinded, long-term follow-up of the phase 2b HVTN503/Phambili study. *Lancet Infect Dis* (2014) **14**:388–96. doi:10.1016/S1473-3099(14)70020-9
18. Hammer SM, Sobieszczyk ME, Janes H, Karuna ST, Mulligan MJ, Groves D, et al. Efficacy trial of a DNA/rAds HIV-1 preventive vaccine. *N Engl J Med* (2013) **369**:2083–92. doi:10.1056/NEJMoa1310566
19. Fauci AS, Marovich MA, Dieffenbach CW, Hunter E, Buchbinder SP. Immune activation with HIV vaccines. *Science* (2014) **344**:49–51. doi:10.1126/science.1250672
20. Johnston MI, Fauci AS. HIV vaccine development-improving on natural immunity. *N Engl J Med* (2011) **365**:873–5. doi:10.1056/NEJMp1107621
21. Lu W, Chen S, Lai C, Guo W, Andrieu J-M. Induction of CD8+ regulatory T cells protects macaques against SIV challenge. *Cell Rep* (2012) **2**:1736–46. doi:10.1016/j.celrep.2012.11.016
22. Van Regenmortel MHV. An oral tolerogenic vaccine protects macaques from SIV infection without eliciting SIV-specific antibodies nor CTLs. *J AIDS Clin Res* (2013) **4**:e112. doi:10.4172/2155-6113.1000e112
23. Esparza J. What has 30 years of HIV vaccine research taught us? *Vaccines* (2013) **1**:513–26. doi:10.3390/vaccines1040513
24. Van Regenmortel MHV, Andrieu J-M, Dimitrov DS, Ensoli B, Hioe CE, Moog C, et al. Paradigm changes and the future of HIV vaccines: a summary of a workshop held in Baltimore on 20 November 2013. *J AIDS Clin Res* (2014) **5**:281. doi:10.4172/2155-6113.1000281
25. Simonton DK. After Einstein: scientific genius is extinct. *Nature* (2013) **493**:602. doi:10.1038/493602a

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Received: 23 June 2014; accepted: 29 June 2014; published online: 14 July 2014.
*Citation: Esparza J and Van Regenmortel MHV (2014) More surprises in the development of an HIV vaccine. Front. Immunol. **5**:329. doi: 10.3389/fimmu.2014.00329*
This article was submitted to HIV and AIDS, a section of the journal Frontiers in Immunology.

frontiers in
IMMUNOLOGY

REVIEW ARTICLE
published: 08 September 2014
doi: 10.3389/fimmu.2014.00417

Challenges in HIV vaccine research for treatment and prevention

***Barbara Ensoli*[1]*, *Aurelio Cafaro*[1], *Paolo Monini*[1], *Simone Marcotullio*[1] and *Fabrizio Ensoli*[2]**

[1] *National AIDS Center, Istituto Superiore di Sanità, Rome, Italy*
[2] *Pathology and Microbiology, San Gallicano Institute, "Istituti Fisioterapici Ospitalieri", Rome, Italy*

Edited by:
Marc H. V. Van Regenmortel, University of Strasbourg, France

Reviewed by:
Sarah Rowland-Jones, John Radcliffe Hospital, UK
Jose Esparza, University of Maryland School of Medicine, USA

***Correspondence:**
Barbara Ensoli, Viale Regina Elena 299, Rome, Italy
e-mail: barbara.ensoli@iss.it

Many attempts have been made or are ongoing for HIV prevention and HIV cure. Many successes are in the list, particularly for HIV drugs, recently proposed also for prevention. However, no eradication of infection has been achieved so far with any drug. Further, a residual immune dysregulation associated to chronic immune activation and incomplete restoration of B and T cell subsets, together with HIV DNA persistence in reservoirs, are still unmet needs of the highly active antiretroviral therapy, causing novel "non-AIDS related" diseases that account for a higher risk of death even in virologically suppressed patients. These "ART unmet needs" represent a problem, which is expected to increase by ART roll out. Further, in countries such as South Africa, where six millions of individuals are infected, ART appears unable to contain the epidemics. Regretfully, all the attempts at developing a preventative vaccine have been largely disappointing. However, recent therapeutic immunization strategies have opened new avenues for HIV treatment, which might be exploitable also for preventative vaccine approaches. For example, immunization strategies aimed at targeting key viral products responsible of virus transmission, activation, and maintenance of virus reservoirs may intensify drug efficacy and lead to a functional cure providing new perspectives also for prevention and future virus eradication strategies. However, this approach imposes new challenges to the scientific community, vaccine developers, and regulatory bodies, such as the identification of novel immunological and virological biomarkers to assess efficacy end-points, taking advantage from the natural history of infection and exploiting lessons from former trials. This review will focus first on recent advancement of therapeutic strategies, then on the progresses made in preventative approaches, discussing concepts, and problems for the way ahead for the development of vaccines for HIV treatment and prevention.

Keywords: HIV-1 vaccine, therapeutics, HAART, functional cure, clinical studies, preclinical and clinical proof-of-concept studies

INTRODUCTION

The HIV epidemic represents one of the major health challenges worldwide, with important social and economical implications for public health. Approximately 34 million people are currently living with HIV, with a total of 24 million accumulated AIDS-related deaths, and 2.6 million new infections (1). With an estimated 6.1 million people living with HIV, South Africa's epidemic remains the largest in the world. Worldwide, the pace of ART roll out to provide universal coverage is outpaced, especially in developing countries, by the number of new infections, rendering the objective almost impossible to achieve (2). Moreover, since ART alone cannot eliminate HIV-1 infection, the therapeutic regimen must be maintained for the lifetime, and this represents a major challenge for the patient (need of strict adherence, poor drug tolerability, drug interactions among antiretroviral agents, and other medications), which may lead to virologic failure and development of drug resistance, and an unbearable economical burden for the National Health Systems. Further, implementation of HAART therapeutic regimens requires a close clinical and laboratory monitoring and the commitment of large human and financial resources with an increasing economic burden for both developing as well as developed countries. This imposes major logistic obstacles to most developing countries, including an insufficient HIV testing, particularly in rural areas, the lack of infrastructures and socio-economical barriers (3–5). Finally, ART alone is unable to eliminate HIV-1 infection. In fact, there is evidence of persistent viral replication in compartments and reservoirs insensitive even to HAART (6). The discrete, though persistent, viral replication as well as HAART-resistant cell-to-cell virus transmission and homeostatic proliferation of infected memory T cells maintain the replenishment of HIV-1 reservoirs (7–16) ensuring virus persistence, while sustaining a residual immune dysregulation, which is associated to chronic immune activation, incomplete restoration of CD4 T cell counts, and lack of replenishment of central memory CD4 and CD8 T cells, which collectively represent the unmet therapeutic needs of ART. In turn, these unmet needs contribute to the residual disease and clinical complications. As a result, HIV-1 infection in the HAART era remains a chronic

progressive disease and is now associated with novel syndromes, termed non-AIDS-associated diseases, including atherosclerosis, cardiovascular diseases, kidney and liver diseases, tumors, early aging, and drug-resistant co-infections (17, 18). These are life-threatening diseases reducing the quality of life of patients still experiencing a high-risk of hospitalization and death. The unmet needs of HAART have a heavy social impact and represent a considerable economic burden for National Health Systems.

Thus, novel, most effective therapeutic strategies are essential to allow a containment of the human, social, and financial resources necessary for the delivery of an effective health care against HIV/AIDS. Indeed, new approaches capable of targeting key pathogenic mechanisms of the virus life cycle, including the establishment and maintenance of virus reservoirs, are urgently needed to circumvent these problems and more effectively attack the virus, either as cART intensification or as an alternative to cART. To this end, novel drugs targeting additional steps in the virus life cycle, gene therapy approaches to render host cells resistant to infection, purging ("shock and kill") strategies to empty viral reservoirs, as well as several different therapeutic immunization approaches are presently being investigated [reviewed in Ref. (19)].

THERAPEUTIC IMMUNIZATION STRATEGIES

In recent years, a growing attention has been given to the development of therapeutic HIV vaccines for treating people already infected with HIV-1. The development of an effective therapeutic vaccine might help at either intensifying ART efficacy, thus fulfilling the ART unmet needs, or, hopefully, at substituting the antiretroviral treatment (**Table 1**). The achievement of either objective might represent a considerable progress beyond the "state of art" of current therapeutic strategies against HIV infection, while ensuring a most favorable cost/efficacy ratio. In addition, an effective therapeutic vaccine may offer a promising alternative strategy to the recurrent failure of preventive HIV vaccines, based on the consideration that it can reduce HIV replication and transmission to healthy individuals. Moreover, therapeutic vaccination may apply to different intervention strategies according to its efficacy, ranging from cART intensification to drug simplification or, for the most effective ones, therapy interruption, or no cART initiation (see **Tables 1** and **2**). Thus, therapeutic vaccination has several advantages on the preventative counterpart, including a rapid and cost–effective proof-of-concept assessment of efficacy even in small phase I/II trials, the possibility to rapidly identify relevant biomarkers of protection, and it may be worth to develop even if not fully effective, since it may be used in association with antiretroviral drugs (see **Tables 1** and **2**). In fact, HIV therapeutic vaccines have been the topics of a conference recently held in Bethesda, MD, USA (20) underscoring the renewed and growing interest to pursue these types of immune interventions, whose potential and feasibility is becoming increasingly appreciated. However, a potential limitation of therapeutic vaccination as compared to the preventative one is that HIV-1 infected individuals may have a reduced immunocompetence, which may hamper both the elicitation and the strength of protective immune responses induced by the vaccine. As immune competence in HIV-1 infected individuals progressively declines over time, therapeutic vaccination early in the course on infection may be required to ensure best efficacy. As discussed later, this might also limit reservoir establishment and promote virus eradication.

Table 1 | Rationale for therapeutic immunization of HIV-infected individuals.

In HIV-infected Drug-Naïve individuals
Delay or block of progression to AIDS or ARV Therapy
In HIV-infected individuals in need of therapy or ARV-treated
1. cART intensification to:
a) Accelerate time-to-response to therapy
b) Block or reduce virus transmission
c) Help reduce reservoir size in patients given intensive ARV in acute infection
d) Solve unmet needs of ART (immune activation, immune defects, and proviral DNA)
2. Drug simplification
3. Therapy interruption after ARV intensification

Although no therapeutic vaccine has been market approved, a growing number of vaccine candidates are being evaluated in phase I/II clinical trials conducted in both naïve and/or ART-treated patients [reviewed in Ref. (21) a comprehensive list of vaccine candidates available at: http://www.pipelinereport.org/sites/g/files/g575521/f/201407/Cure%20Immune%20Based%20and%20Gene%20Therapies.pdf]. Here, we briefly review the most representative of the diverse approaches undertaken.

InnaVirVax – (a spin-off of INSERM, Evry, France) is developing VAC-3S, a vaccine constructed to induce a humoral immune response against a highly conserved region of the envelope protein gp41 of HIV-1 known as 3S. The 3S domain has been shown to induce the expression of NKp44L on uninfected CD4 T cells, rendering them susceptible to lysis by $NKp44^{+}$ activated NK cells, thus contributing to the massive T cell loss, which far exceeds the number of HIV-infected lymphocytes (22). Upon promising data in monkeys (23), it has recently been announced the start of a randomized, double-blind, placebo-controlled phase II study in 90 cART-suppressed adult subjects, with the primary endpoint being the induction of anti-3S antibodies, while overall tolerance and clinical safety, together with a comprehensive evaluation of the immunological end-points, inflammatory biomarkers, and vaccine's immunogenic characteristics, represent the secondary end-points (ClinicalTrials.gov Identifier:NCT02041247).

Bionor Pharma (Oslo, Norway) is developing Vacc-4x, a peptide-based vaccine consisting of four synthetic peptides based on the HIV-1 p24 protein, injected multiple times intradermally together with GM-CSF. The results from a double-blind, randomized, phase II study conducted in 135 patients on effective cART showed that Vacc-4x is sae, immunogenic, and contributes to viral load reduction after cART interruption. However, the proportion of participants resuming cART before the end of the study and the CD4 T cell counts recorded during the treatment interruption showed no benefit of vaccination. In addition, it requires a

Table 2 | HIV/AIDS preventative and therapeutic vaccine . . . the continuum.

Status	HIV-negative	Asymptomatic	At cART initiation	Upon cART initiation		
				Vaccine efficacy		
				Low	⟶	High
Goal	Prevention of infection	Block of progression (no cART need)	Faster and more efficient response to therapy	cART intensification	cART simplification	cART interruption
		(functional cure, eradication)				
Primary (short-term) end-points	HIV test Viral load CD4 counts	CD4 counts Viral load Time to cART initiation	CD4 counts Viral load Time to infection control	CD4 counts Viral load		
Secondary (long-term) end-points	None	Proviral DNA (Integrated, non-integrated, total) Neutralization of Tat/Env entry in DCs Markers of immune activation and reconstitution				

long and complex vaccination schedule while providing a limited impact on viral load (24).

SEEK (London, UK) is developing PepTcell, a T cell epitope HIV vaccine consisting of synthetic peptides derived from the conserved regions of Vpr, Vif, Rev, and Nef. A randomized, placebo-controlled, dose finding Phase Ib/II trial was recently completed in 55 HIV-positive volunteers. After just a single subcutaneous injection, a significant reduction in viral load was observed in a minority of vaccinees who had received the high dose and had mounted both B and T cell responses to the vaccine (25).

FIT Biotech (Tampere, Finland) is developing GTU® MultiHIV multi-gene, a vaccine based on six viral HIV proteins. The vaccine (clade B) has been tested in 60 untreated, asymptomatic, HIV-1 subtype C infected adults enrolled in a single-blinded, placebo-controlled Phase II trial in South Africa. The vaccine was safe and well tolerated. Significant declines in plasma HIV-RNA load and increases in $CD4^+$ T cell counts were observed in the vaccine group compared to placebo, which were more pronounced after intramuscular (IM) administration (26).

Similarly, Profectus Biosciences (Baltimore, MD, USA) is developing TheraVax, a multi-antigen HIV vaccine in which a plasmid DNA encoding Env, Gag, Pol, Nef, Tat, and Vif is administered by electroporation, in combination with interleukin-12 plasmid DNA followed by a boost with same the antigens vectored by a recombinant vesicular stomatitis virus (rVSV) delivered intramuscularly. A randomized, double-blind, placebo-controlled phase I study in cART-treated patients ($n = 50$) has started in USA and results are expected by November 2016 (ClinicalTrials.gov Identifier: NCT01859325).

Genetic Immunity (Budapest, Hungary) is developing DermaVir, a DNA vaccine encoding 15 HIV proteins administrated by skin patches. A randomized, placebo-controlled, dose-ranging Phase II study in 36 HIV-infected individuals naïve to cART confirmed that the vaccine, administered three times, 6 weeks apart, is safe and immunogenic (27). A 0.5 Log_{10} reduction of plasma RNA copies per milliliter was seen in the arm immunized with the intermediate DermaVir dose (0.4 mg). However, no amelioration of $CD4^+$ T cell counts was recorded (27).

GeoVax (Atlanta, GA, USA) is developing MVA/HIV62B, a two components' vaccine: a recombinant DNA vaccine and a recombinant MVA (modified vaccinia Ankara) vaccine. Both produce non-infectious virus-like particles displaying HIV clade B Env, Gag, and Pol proteins. An open label phase I study in HIV-1 infected adults on successful ART who initiated therapy within 18 months from seroconversion ($n = 9$) is ongoing (ClinicalTrials.gov Identifier: NCT01378156).

Theravectys SAS (a spin-off of the Pasteur Institute, Paris, France) is developing THV01, a vaccine based on lentiviral vectors (01 and 02), both encoding HIV-1 Clade B Gag, Pol, and Nef proteins and exploiting the HIV DNA flap sequence, which permits the nuclear import of HIV in non-dividing cells, including dendritic cells (DCs), thus optimizing antigen immunogenicity. Upon promising efficacy data in macaques (28), Theravectys recently announced the initiation of a Phase I/II randomized, double-blind, placebo-controlled trial of 36 patients in 6 clinical centers aimed at assessing the safety and tolerance of the vaccine and measure the quality and intensity of the induced immune response, which, in perspective, should allow therapy interruption (ClinicalTrials.gov Identifier: NCT02054286).

Argos Therapeutics (Durham, NC, USA) is developing tailor-made vaccines in that autologous DCs, loaded *ex vivo* with RNA encoding four (Gag, Nef, Rev, and Vpr) of the patient's own HIV antigens plus CD40L, are reinjected into the patient intradermally, four times, 4 weeks apart. Results from the Phase IIa ($n = 24$) study indicate delay of cART resumption in treated subjects, but no improvement of $CD4^+$ T cell counts (29).

Of note, none of these vaccine candidates is aimed at intensifying HAART efficacy in order to attack the virus reservoirs and restore the immune homeostasis. To this aim, the Italian National AIDS Center is developing a vaccine based on the biologically active HIV Tat protein. Results from phase I preventative (ISS P-001, ClinicalTrials.gov identifier: NCT00529698) and therapeutic (ISS T-001, ClinicalTrials.gov identifier: NCT00505401) studies have indicated that the Tat vaccine is safe and immunogenic (30–32) and more recently results from a randomized phase II trial (ISS T-002) in virologically suppressed HAART-treated subjects (ClinicalTrials.gov NCT00751595) indicate that Tat vaccination exerts a positive impact on immune activation and T and B cell dysregulation [Ref. (33, 34) and Ensoli et al., manuscript submitted], confirming the role of Tat in the pathogenesis of the HAART unmet needs. Tat immunization induced a restoration of $CD4^+$ and $CD8^+$ T cell number and functional central memory T cell subsets, of B and NK cell number and a reduction of immune activation as compared to subjects under effective HAART (33). Of importance,

Tat immunization induced a statistically significant reduction of blood HIV-1 DNA load [Ref. (34) and Ensoli et al., manuscript submitted]. Effects were greatest with Tat 30 μg, given three times at monthly intervals, and under Protease Inhibitors (PI)-based regimens, with a predicted 70% HIV-1 DNA decay after 3 years from vaccination and a half-life of 88 weeks. HIV-1 DNA decay was associated with anti-Tat Abs and neutralization of Tat-mediated entry of oligomeric Env in DCs, which predicted HIV-1 DNA decay. A phase II randomized, placebo-controlled clinical trial (ISS T-003, ClinicalTrials.gov Identifier: NCT01513135) of the Tat vaccine has just been completed in South Africa in 200 HAART-treated individuals and the results are expected by the end of 2014. Strategies are in development for phase III registrative trials.

More recently, also Biosantech SA – France (a spin-off of ANRS) started developing a vaccine based on a synthetic form of Tat derived from Tat Oyi, an attenuated clade B HIV field isolate. Based on results in monkeys (35), Biosantech recently announced the start of a therapeutic phase I study in 48 patients under cART. The strategy is aimed to therapy interruption.

As an alternative to therapeutic vaccines strategy, Sangamo BioSciences (Richmond, CA, USA), is developing SB-728-T, an *ex vivo* gene therapy approach by which $CD4^+$ T cells drawn from HIV-infected patients are modified *ex vivo* to disrupt the CCR5 gene in autologous $CD4^+$ T cells, expanded, and reinfused to the patient. Results from a phase I study indicate that both CCR5 alleles have to be disabled to make the treatment effective (36). However, the complexity and costs of this approach, together with serious safety issues represent a major disadvantage, rendering the immune therapy, even if it was effective, accessible only to a small fraction of the HIV population (37). Nevertheless, alternative strategies to render the patient's cells resistant to infection are being developed and may turn out to be more feasible than at present (38–40).

Altogether, the above studies demonstrate that the HIV/AIDS therapeutic vaccine field is rapidly expanding and portraits a substantial variety of approaches, which differ sensibly in many aspects, the most relevant being the antigen chosen (unlike preventative vaccines, regulatory and accessory genes are frequently targeted; in some cases almost the entire HIV genome is targeted), and the delivery systems, which range from simple subcutaneous, intradermal, or intramuscular vaccine administration to reinfusion of autologous DCs loaded *ex vivo* with the selected antigen(s), or, for the gene therapy approaches, of genetically modified autologous target cells.

As compared to preventative approaches, the therapeutic setting provides the unique opportunity to evaluate vaccine efficacy in a more rapid and convenient manner, hopefully speeding up the identification and development of effective vaccine candidates. However, key clinical end-points and appropriate virological and immunological biomarkers to properly assess the therapeutic efficacy in more advanced trials still need to be established and agreed upon. To address these issues is a priority to grant advancement of therapeutic, and possibly preventative, vaccines.

PREVENTATIVE VACCINATION STRATEGIES

Despite almost 30 years of efforts, a preventative HIV vaccine is still lacking and only four types of HIV vaccines have been tested in six HIV vaccine efficacy trials so far (41, 42).

The first efficacy trials were conducted in high-risk populations immunized with a mixture of monomeric form of gp120 Env from two different clade B (VAX004) or from clade B and E (VAX003). Both vaccines, aimed at inducing neutralizing Abs (NAbs), failed to prevent infection (43, 44) and investigators turned their attention and hopes to vaccines aimed at inducing CD8 T cell responses.

The MRK rAd5 vaccine consisted of HIV-1 Gag, Pol, and Nef delivered by three different recombinant adenovirus 5 (rAd5) vectors and aimed at inducing protective $CD8^+$ T cell responses. The phase IIb "test-of-concept" STEP trial (also termed HVTN 502 or Merck V520-023 study) was stopped due to evidence of enhanced risk of acquisition of infection, especially in those uncircumcised and with pre-existing antibodies to the vector. Because of this serious safety concern, the companion Phambili trial (HVTN 503) conducted in South Africa was also halted (45, 46). A very recent analysis confirmed that also in the Phambili trial the rate of acquisition of HIV infection was higher among vaccinees, especially during the long-term follow-up, for unknown reasons, although early unblinding due to trial stop might have influenced risk behavior (47). Of note, the temporal windows of increased risk of acquisition were the opposite in HVTN 502 (early after vaccination) and HVTN 503 (late after vaccination), underscoring the complexity of the factors involved, including differences in the risk populations targeted. A detailed analysis of these rAd5-based trials showed a strong activation of Ad5-specific (but not HIV-specific) CD4 T cells in the gut mucosa of vaccinees, which may explain their enhanced susceptibility to infection (48), a finding reproduced in the monkey model (49). Nevertheless, *post hoc* "sieve" analysis of breakthrough infections demonstrated that the vaccine had exerted some immune pressure, as indicated by the appearance of virus-escape mutants (50). However, the immune pressure was against HIV-1 epitopes contained predominantly in highly variable regions infrequently targeted in the course of the natural infection, domains which can presumably tolerate escape mutations (51). Thus, vaccines aimed at generating $CD8^+$ T cell responses may still be a valid option, provided that better strategies and vectors are identified.

In fact, another rAd5-based preventative phase III trial was stopped in 2013 due to evident lack of efficacy (52). The HVTN 505 vaccine consisted of HIV-1 Gag, Pol, Nef, and three different Env from clade A, B, and C, delivered as DNA for priming followed by boosting with rAd5 vectors coding for all but Nef antigen. Despite these rAd5 vectors were less immunogenic than those used in the STEP trial and despite individuals seropositive for rAd5 were excluded from the trial, breakthrough infections were slightly higher among vaccinees as compared to placebo, casting serious doubts on whether to proceed further with adenoviral vectors (53) and, more in general, with vectors whose immune activating properties may exceed the capability to induce protective immune responses, thus favoring, rather than blocking, susceptibility to infection (48).

So far, only one efficacy trial has provided some evidence of low and transient (60% at 12 months but 31% at 42 months) protection from acquisition of infection. The RV144 Thai trial was an Env-based vaccine consisting of a priming with the $CD4^+$ T cell-stimulating ALVAC canarypox expressing HIV-1 gag/pr/gp41/gp120 followed by the VAX004 gp120 Env of B and

E clade, the very same used in the AIDSVAX trial reported above. Intriguingly, protection correlated with titers of IgG against the V1V2 loop of Env, which were not neutralizing but mediating ADCC, whereas, when present, high titers of IgA against the C1 domain of Env actually abolished the IgG effect by interfering with the IgG binding to V1V2 (54). Of note, mostly low to medium-risk individuals were enrolled in the RV144 Thai trial, and vaccine efficacy dropped to 3.7% when only high-risk individuals were considered (55). Nevertheless, this was considered an important step ahead in vaccine development and stemming from these results, several new trials have been designed to reproduce and possibly increase the efficacy (56).

It is worth considering that the Env-protein based vaccines tested so far in clinical trials have utilized gp120 Env monomers or a truncated form of gp160 (HVTN 505), although the spikes present on the infectious virion are constituted by trimers of gp160, which differ from monomeric gp120 Env in terms of antigenic properties and conformational epitopes displayed (57). This further emphasizes the concept that vaccine design should be more "pathogenesis-driven" in order to effectively target key virus molecules, a notion to carefully consider in future vaccine development. An oligomeric Env that closely resembles the native protein has been recently generated, which may represent a better immunogen and a useful tool to detect valuable anti-Env Abs (58).

On the other hand, approaches based on vaccines aimed at inducing protective $CD8^+$ T cell responses only (HVTN 502 and 503) or in association to anti-Env antibodies (HVTN 505) have been largely disappointing. However, recent data obtained in the macaque model provided some encouraging results, although not necessarily easily transferable to human. This type of vaccine it is not expected to protect from infection acquisition, but rather to contain infection (i.e., low to undetectable plasma viral load and no $CD4^+$ T cell loss), preventing progression to disease as well as virus transmission (**Figure 1**).

Strong control and apparent clearance of infection upon mucosal challenge with the pathogenic SIVmac239 was obtained with a replication competent simian cytomegalovirus (CMV) vector engineered to express SIV Gag, Tat, Rev, Nef, Env, and Pol (59–61). This strategy induced effector memory CD8 T cells which localized in peripheral tissues, including the mucosal portal of entry of the virus, thus providing the opportunity for the CTLs to attack the virus prior to dissemination. In fact, protected macaques experienced a reduced peak viremia, which rapidly subsided to undetectable levels, no CD4 T cell loss, no seroconversion, poor establishment of virus reservoirs in lymphoid tissues and effector tissues, eventually disappearing, a finding consistent with eradication of the infection (59–61). Protection appeared to be mediated by effector T cells present at the site of infection, although they were able to protect only 50% of the vaccinated monkeys, with the other half experiencing an infection comparable to controls (59–61). Intriguingly, CD8 T cells the effectors were restricted prevalently by class II rather than class I MHC antigens, and responses were very broad and persistent, features that appear to be peculiar to the replication competent modified CMV vector used (62). Besides underscoring the importance of the vector in the response to a vaccine, which also poses safety issues that will have to be exhaustively addressed before testing in human, this strategy provides a further indication that, to be effective, T cell responses have to be already in place at the portal of entry when the virus attacks. Still, if this defense line is overcome, the infection proceeds unaffected, indicating that effector T cells are necessary but not always sufficient to afford protection.

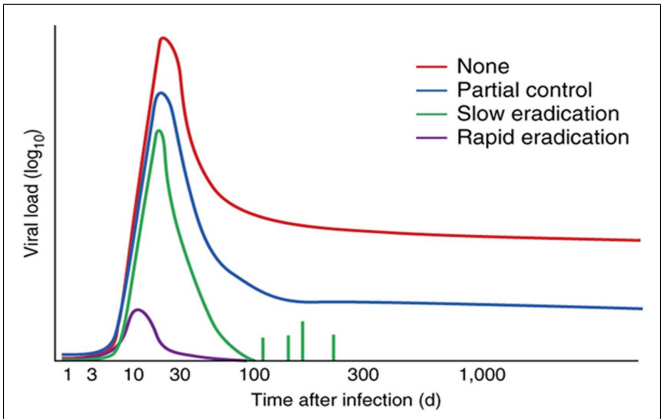


FIGURE 1 | Control of HIV-1 by vaccines that stimulate CTLs. Effect of various T cell-stimulating vaccines (key) on viral load over time (with infection on day 0) during natural infection with HIV or SIV, showing the decrease in viral load achieved without a vaccine (none), by CTL responses [partial control; as in Ref. (92), for example], by the RhCMV vaccine (slow eradication) (60, 61) and by a hypothetical vaccine that targets the virus at the site of infection (rapid eradication). Reproduced with permission from Ref. (61).

While the above strategy was aimed at inducing a specific type of effector cell, others are focusing on the selection of relevant epitopes [reviewed in Ref. (63)]. Mosaic antigens (64) and conserved antigens (65, 66) represent two potential strategies to address the challenges of global HIV-1 diversity (**Figure 2**). The first takes advantage of bioinformatics to generate mosaic antigen to cover most of the variants of each epitope displayed by circulating viruses with the aim of increasing the breadth of humoral and cellular immune responses, whereas conserved antigens aim to focus cellular immune responses on the most conserved viral sequences. Although the mosaic antigen approach seems more promising and has shown some efficacy in preclinical models (67), both strategies needs major improvements (better targeting of relevant epitopes, superior immunogenicity, durable immunity, and identification of correlates of protection) prior to progress to clinical trials.

A further approach based on a "pathogenetic" assumption, and thus aimed at inducing effective immune responses against a key HIV virulence factor, has been developed by targeting the HIV Tat protein. Tat vaccination represents an example of a "pathogenic-driven" intervention potentially effective for both preventative and therapeutic objectives, since it is aimed at blocking virus transmission/spreading. The rationale is based on the evidence that HIV-1 Tat, which is necessary for HIV gene expression, replication, and cell-to-cell transmission, appears also to be critical in the initial steps of virus acquisition. In fact, it has been recently shown that Tat, which is present on virus particles, binds to Env spikes promoting HIV infection of DCs and spreading to T lymphocytes even in the presence of anti-Env NAbs and that

anti-Tat Abs are necessary to restore neutralization (**Figure 3**) (68). This evidence provides some explanation to the repeated failure of preventative vaccines based solely on Env and indicates that Tat may represent an optimal target for preventative interventions either alone or in combination with oligomeric Env.

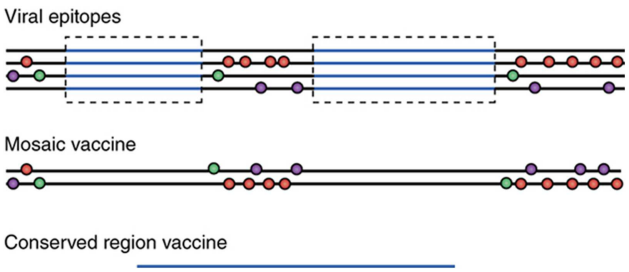


FIGURE 2 | Vaccines that deal with HIV-1 variability. Construction of vaccines based on viral sequences in four viral isolates (top; simplified representation): horizontal lines indicate viral sequences; circles indicate sites of greatest variability between isolates (and potential escape mutations from CTL pressure; there may be more than two alternative sequences at each spot); and blue lines indicate regions of relative conservation (although in reality no region of HIV-1 is invariant). The mosaic vaccine (middle) is constructed to include the most common variants from the isolates in as few strands as possible while conserving naturally occurring sequence stretches. In the conserved region-containing vaccine (bottom), the relatively conserved regions (blue) are excised and then are "stitched" together (which creates an unnatural junctional region). The regions typically vary from 30 to 120 amino acids in length. Reproduced with permission from Ref. (63).

Preclinical studies in cynomolgus monkeys have shown that immunization with a biologically active Tat protein or *tat* DNA is safe, elicits a broad and specific immune response and, most importantly, induces a long-term protection against infection with a highly pathogenic SHIV-89.6P encoding Tat of HIV-1, which rapidly causes AIDS and death in these monkeys (69, 70). Both Tat protein and *tat* DNA elicited memory Tat-specific Abs, $CD4^+$, and $CD8^+$ T cell responses in protected monkeys, which did not show signs of systemic infection throughout a 104-week follow-up or even upon a further boosting with tetanus toxoid (71), providing clear evidence of long-term containment of virus replication and spread in blood and tissues (72). A retrospective analysis of 112 Mauritian cynomolgus macaques from different preclinical trials, vaccinated ($n = 67$) or not ($n = 45$) with Tat and challenged with the SHIV-89.6P, showed that vaccination induced a significant reduction of the rate of infection acquisition at 10 MID_{50} ($P < 0.0001$), and contained acute $CD4^+$ T cell loss at 15 MID_{50} ($P = 0.0099$). Of importance, vaccination also contained $CD4^+$ T cell depletion ($P = 0.0391$) during chronic infection, irrespective of the challenge dose (73).

In a different approach, rhesus macaques primed mucosally with two replicating adenoviruses expressing HIV-1 Env and Tat, respectively, and boosted with the Tat and Env proteins became all infected following high dose intravenous SHIV-89.6P challenge. However, the Tat/Env vaccinated monkeys reduced chronic

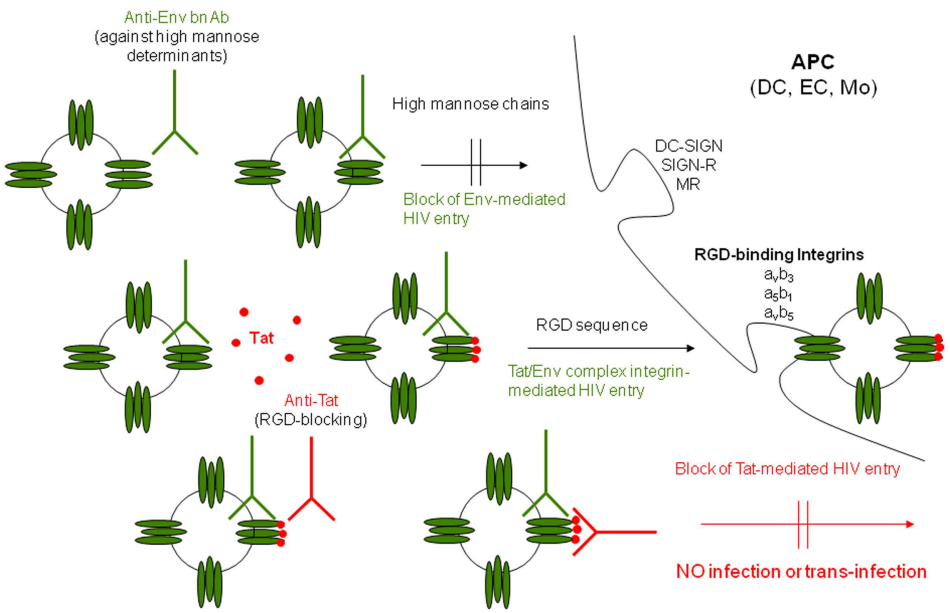


FIGURE 3 | Outcome of DC infection in the absence or presence of Tat, anti-Env, and/or anti-Tat antibodies. Tat redirects HIV to RGD-binding integrins evading neutralization by anti-Env Abs and both anti-Env and anti-Tat Abs are required to block infection. Extracellular Tat released by infected neighbor cells binds to trimeric Env on HIV, decreasing recognition of C-type lectin receptors and promoting engagement of RGD-binding integrins, which are expressed by inflammatory DCs, macrophages, and endothelial cells (ECs) present at the site of infection. As a result, virions escape neutralization by anti-Env Abs directed against high mannose determinants and enters target cells upon binding to RGD-binding integrins. Anti-Tat Abs neutralize this binding, preventing virus entry through RGD-binding integrins. DC-SIGN: dendritic cell-specific intercellular adhesion molecule-3-grabbing non-integrin; SIGN-R: homolog of DC-SIGN present on ECs; MR: mannose receptor [modified from Ref. (68)].

viremia by four logs as compared to controls ($P < 0.0001$). Of note, control of infection correlated with Tat and Env binding Abs (74).

Protection or containment of infection were also observed in cynomolgus macaques co-immunized with HIV-1 Tat and Env proteins and challenged intrarectally with a high dose (70 MID_{50}) of the R5-tropic $SHIV_{SF162P4cy}$ (68). In this case, the macaques had been primed twice intranasally with HIV-1 Tat and Env, given together with the LT-K63 mucosal adjuvant, and then boosted subcutaneously with Tat plus Env in Alum. No infection or a statistically significant reduction of viral loads and proviral DNA were observed in the vaccinated monkeys as compared to controls. Notably, proviral load in the inguinal lymph nodes was significantly lower in vaccinated monkeys as compared to controls, whereas it did not differ significantly in rectal biopsies, strongly suggesting the block of virus dissemination from the portal of entry (68).

In a further approach, the sterilizing immunity or control of infection observed in rhesus macaques immunized with a multicomponent vaccine (multimeric HIV-1 gp160, HIV-1 Tat, and SIV Gag-Pol particles) delivered either systemically or mucosally and challenged orally or intrarectally with the C clade r5-tropic SHIV-1157ip correlated only with anti-Tat Abs against the N-terminus of Tat, as determined by a novel biopanning strategy which, using recombinant phages encoding random peptide libraries, allows a complete and unbiased profiling of the antibody repertoire and identification of epitopes associated with vaccine protection (75).

These studies strongly suggest that the induction of anti-Tat Abs may be key to achieve protective immunity against HIV/AIDS. In this regard is worth to note that in natural HIV infection, anti-Tat Abs are produced by only a small fraction of individuals (76, 77), while, in contrast, high Ab titers are produced against all other viral products (78). The reason for such a limited anti-Tat Ab response is unclear. However, when present, anti-Tat Abs correlate with the asymptomatic state and lower or no disease progression (79–83). In particular, a cross-sectional and longitudinal study, on 252 HIV-1 seroconverters, with a median follow-up time of 7.2 years, indicated that the presence of anti-Tat Abs is predictive of a slower progression to AIDS or immunodeficiency (83). Progression was faster in persistently anti-Tat Ab-negative than in transiently anti-Tat Ab-positive subjects, whereas no progression was observed in individuals persistently anti-Tat-Ab positive (83). Thus, anti-Tat Abs may have a protective role and represent a predictive biomarker of slower progression to AIDS.

The effects of anti-Tat Abs on the immunological, virological, and clinical outcome of HIV-infected subjects were recently assessed in a prospective observational study (ISS OBS T-003, ClinicalTrials.gov NCT01029548) conducted in asymptomatic drug-naïve HIV-infected adult volunteers (84). A significant association between the presence of anti-Tat Abs and a slower disease progression was found. In particular, anti-Tat Ab-positive patients showed a remarkable preservation of $CD4^+$ T cells and containment of viral load for the entire follow-up (3 years), and no individuals with high levels of anti-Tat Abs initiated HAART during follow-up (84). Of note, the association of increasing anti-Env IgG titers with a lower risk of starting HAART occurred only in the presence of anti-Tat Abs, suggesting that anti-Tat and anti-Env Abs combined have increased HIV neutralizing effects by blocking the Tat/Env complex formation and virus entry, as shown earlier both *in vitro* and *in vivo* (68). Thus, both anti-Tat and anti-Env Abs appear to be required to efficiently block HIV disease progression. In contrast, anti-Env or anti-Gag Ab titers had no significant effects on $CD4^+$ T cell counts and viral load in patients naïve to therapy with or without anti-Tat Abs (84).

Of importance, the results of a phase I safety and immunogenicity clinical trial (ISS P-001, ClinicalTrials.gov identifier: NCT00529698), indicate that Tat immunization is safe in HIV-negative healthy individuals and highly immunogenic, as it induced high titers of cross-clade, neutralizing anti-Tat Abs [(30–32), and unpublished results]. More recently a phase I, open label trial was conducted to assess the safety and immunogenicity in HIV uninfected healthy adult volunteers of a preventative vaccine based on the association of recombinant HIV-1 biologically active Tat and oligomeric Env deleted in the V2 region proteins (ISS P-002, ClinicalTrials.gov identifier: NCT01441193), which is currently under analysis.

DISCUSSION AND PERSPECTIVES

An innovative targeting for HIV vaccine development should exploit lessons from former trials and pathogenetic mechanisms, taking advantage of the natural history of infection in humans (epidemiology, spontaneous control of infection). In fact, they provide valuable information: either the vaccine did not induce the responses desired and the immunization strategy has to be improved or changed, or it did it, but the responses were not protective, and the immunogen must be redesigned or the target antigen changed.

So far, HIV vaccine design based on structural knowledge has not been successful, nor have been empirical vaccines, reinforcing the concept that a rational pathogenetic approach must be undertaken to identify key virulence factors to be exploited for vaccine targeting. A "pathogenesis-driven" approach should be aimed at targeting key viral products responsible of virus transmission, activation and maintenance of virus reservoirs. Stemming from these considerations one may then argue whether a preventative vaccine should actually be different from a therapeutic one: a preventative vaccine capable of blocking virus entry and transmission should also be able to block virus spread within the infected host and, vice versa, a therapeutic vaccine targeting key steps of the virus life cycle and/or replication may rapidly control intra-host spreading after acquisition and hopefully eradicate the infection. In both cases, the rationale for vaccine design is to target key HIV virulence factor(s) required for virus entry/transmission and/or spreading. This is true in both healthy and already infected people, even those on suppressive HAART. In fact, in considering these two extremes, it appears that the viral dynamics is very similar. For infection acquisition, the virus must find/induce optimal conditions for the infection to occur, as indicated by the low transmission rate and the existence of individuals who remain uninfected despite being repeatedly exposed (85, 86). In fact, during the initial steps of viral infection the virus needs to overcome the mucosal barrier and to find proper target cells such as DCs, macrophages, activated CD4 T cells, to rapidly replicate and spread (87, 88). The presence of intraepithelial DCs, capable of sampling the "outside" and to rapidly bring and transfer the virus to neighbor CD4 T

cells and activate them seems to be pivotal to infection establishment (87, 88). Accordingly, inflammation, immune activation and mucosal lesions (mostly due to other sexually transmitted infection, STI) enormously enhance sexual transmission (89, 90). From this first focus of infected cells and through discrete rounds of cryptic infection, virus establishes itself and initiates a productive infection. Strikingly, evidence of ongoing residual virus replication or reactivation have been reported even during suppressive HAART, which somewhat recapitulate and mimic the difficulties HIV encounters in the primary infection (91) Thus, understanding and blocking the mechanism(s) of virus transmission in primary as well as in chronic infection, in individuals either asymptomatic and naïve to drug or on HAART, will conceivably lead, respectively, to protection from infection and to virus eradication.

Thus, a "pathogenic-driven" strategy targeting a key virulence factor might be effective in both the preventative (healthy people) and the therapeutic approach (either in subjects naïve to drugs or on cART), by acting with the same mechanisms, in preventing/controlling HIV infection/progression to disease. The outstanding control and apparent eradication of infection conferred by CTL responses elicited upon preventative immunization with a CMV-vectored vaccine in a macaque model (58–60) is perhaps one of the best examples of a successful prevention obtained with a strategy aimed at inducing cellular responses (i.e., effector memory CTL) known to be associated to non-sterilizing immunity. Therefore, the distinction between preventative and therapeutic vaccine concepts and strategies should not necessarily be considered in terms of the development of different approaches, but rather in terms of targeting distinct populations (i.e., uninfected individuals vs. HIV-infected subjects) (**Table 2**). In considering this, it should be noted that the conduction of therapeutic trials may prove very useful and cost–effective in providing a first proof-of-concept of efficacy of a vaccine design and better define specific end-points and laboratory biomarkers to be assessed, before advancing to the very expensive and time-consuming preventative trials (**Table 2**). To these goals, novel immunological and virological biomarkers (in addition to viral load and CD4 T cell counts) should be taken into consideration in trial design in order to assess the achievement of efficacy end-points (i.e., assessment of functional T and B cell subsets, cellular and biochemical immune activation biomarkers, proviral DNA in reservoirs, cell-to-cell virus transmission, and virus neutralization). This approach imposes new challenges to the scientific community, vaccine developers and regulatory bodies, which require new paradigms and a new "way ahead."

CONCLUSION

New paradigms must be applied to develop efficacious preventative/therapeutic intervention strategies against HIV. These new concepts may also serve to combat epidemics by other agents. "Pathogenesis-driven" approaches should be considered with an open-minded attitude and should constitute the basis for a rationale vaccine design, taking also into account that structures do not always translate in immunogenicity and immunogenicity does not always translate in efficacy. The disappointing results from efficacy trials together with difficulties in translating preclinical studies to clinical trials, including, but not limited to, the uncertain predictivity of the results obtained in non-human primate models, also indicate that vaccine candidates should first be tested in infected individuals. This will provide a solid proof-of-concept to advance to the very expensive and time-consuming preventative trials. Further, new vaccine concepts and clinical trial designs should be considered and supported with proper funding. This also requires new methods for evaluation of projects where innovation is a key indicator. In fact, new vaccine design may require different endpoints and biomarkers of efficacy as well as new testing for safety. Thus, regulatory bodies must be involved at an early stage of development and should be available to discuss the proper planning and conduction of innovative approaches.

ACKNOWLEDGMENTS

The authors thank A. Tripiciano, S. Bellino, O. Longo, V. Francavilla, O. Picconi, and C. Sgadari for helpful discussion, and F. Cammisa and G. Fornari Luswergh (National AIDS Center, Istituto Superiore di Sanità, Rome, Italy) for support to study management and editorial assistance, respectively; S. De Menna, A. Biondi, S. Tobelli, and F. Fedeli (National AIDS Center, Istituto Superiore di Sanità, Rome, Italy) for administrative support.

REFERENCES

1. UNAIDS. *Global Report: UNAIDS Report on the Global AIDS Epidemic 2013.* Geneva: WHO (2013).
2. UN Secretary-General. *Uniting for Universal Access: Towards Zero New HIV Infections, Zero Discrimination and Zero AIDS-Related Deaths.* UNAIDS report, Nairobi (2011). Available from: http://www.unaids.org/en/media/unaids/contentassets/documents/document/2011/A-65-797_English.pdf
3. Boyer S, March L, Kouanfack C, Laborde-Balen G, Marino P, Aghokeng AF, et al. Monitoring of HIV viral load, CD4 cell count, and clinical assessment versus clinical monitoring alone for antiretroviral therapy in low-resource settings (Stratall ANRS 12110/ESTHER): a cost-effectiveness analysis. *Lancet Infect Dis* (2013) **13**(7):577–86. doi:10.1016/S1473-3099(13)70073-2
4. Vassall A, Remme M, Watts C, Hallett T, Siapka M, Vickerman P, et al. Financing essential HIV services: a new economic agenda. *PLoS Med* (2013) **10**(12):e1001567. doi:10.1371/journal.pmed.1001567
5. Leclerc-Madlala S. Silver bullets, glass beads, and strengthening Africa's HIV response. *Lancet* (2014) **383**(9924):1203–4. doi:10.1016/S0140-6736(14)60593-6
6. Sigal A, Kim JT, Balazs AB, Dekel E, Mayo A, Milo R, et al. Cell-to-cell spread of HIV permits ongoing replication despite antiretroviral therapy. *Nature* (2011) **477**(7362):95–8. doi:10.1038/nature10347
7. Chomont N, El-Far M, Ancuta P, Trautmann L, Procopio FA, Yassine-Diab B, et al. HIV reservoir size and persistence are driven by T cell survival and homeostatic proliferation. *Nat Med* (2009) **15**:893–900. doi:10.1038/nm.1972
8. Chun TW, Justement JS, Murray D, Hallahan CW, Maenza J, Collier AC, et al. Rebound of plasma viremia following cessation of antiretroviral therapy despite profoundly low levels of HIV reservoir: implications for eradication. *AIDS* (2010) **24**(18):2803–8. doi:10.1097/QAD.0b013e328340a239
9. Yukl SA, Shergill AK, McQuaid K, Gianella S, Lampiris H, Hare CB, et al. Effect of raltegravir-containing intensification on HIV burden and T-cell activation in multiple gut sites of HIV-positive adults on suppressive antiretroviral therapy. *AIDS* (2010) **24**(16):2451–60. doi:10.1097/QAD.0b013e32833ef7bb
10. Cameron PU, Saleh S, Sallmann G, Solomon A, Wightman F, Evans VA, et al. Establishment of HIV-1 latency in resting CD4+ T cells depends on chemokine-induced changes in the actin cytoskeleton. *Proc Natl Acad Sci U S A* (2010) **107**(39):16934–9. doi:10.1073/pnas.1002894107
11. Buzón MJ, Massanella M, Llibre JM, Esteve A, Dahl V, Puertas MC, et al. HIV-1 replication and immune dynamics are affected by raltegravir intensification of HAART-suppressed subjects. *Nat Med* (2010) **16**(4):460–5. doi:10.1038/nm.2111

12. Sigal A, Baltimore D. As good as it gets? The problem of HIV persistence despite antiretroviral drugs. *Cell Host Microbe* (2012) **12**(2):132–8. doi:10.1016/j.chom.2012.07.005
13. Johnson TP, Patel K, Johnson KR, Maric D, Calabresi PA, Hasbun R, et al. Induction of IL-17 and nonclassical T-cell activation by HIV-Tat protein. *Proc Natl Acad Sci U S A* (2013) **110**(33):13588–93. doi:10.1073/pnas.1308673110
14. Chun TW, Fauci AS. Viral persistence in HIV infection: much known, much to learn. *J Infect Dis* (2013) **208**(9):1356–8. doi:10.1093/infdis/jit455
15. Katlama C, Deeks SG, Autran B, Martinez-Picado J, van Lunzen J, Rouzioux C, et al. Barriers to a cure for HIV: new ways to target and eradicate HIV-1 reservoirs. *Lancet* (2013) **381**(9883):2109–17. doi:10.1016/S0140-6736(13)60104-X
16. Buzon MJ, Martin-Gayo E, Pereyra F, Ouyang Z, Sun H, Li JZ, et al. Long-term antiretroviral treatment initiated in primary HIV-1 infection affects the size, composition and decay kinetics of the reservoir of HIV-1 infected CD4 T cells. *J Virol* (2014) **88**(17):10056–65. doi:10.1128/JVI.01046-14
17. Phillips AN, Neaton J, Lundgren JD. The role of HIV in serious diseases other than AIDS. *AIDS* (2008) **22**(18):2409–18. doi:10.1097/QAD.0b013e3283174636
18. Marin B, Thiébaut R, Bucher HC, Rondeau V, Costagliola D, Dorrucci M, et al. Non-AIDS-defining deaths and immunodeficiency in the era of combination antiretroviral therapy. *AIDS* (2009) **23**(13):1743–53. doi:10.1097/QAD.0b013e32832e9b78
19. Hoxie JA, June CH. Novel cell and gene therapies for HIV. *Cold Spring Harb Perspect Med* (2012) **2**(10):ii:a007179. doi:10.1101/cshperspect.a007179
20. Fisher AK, Voronin Y, Jefferys R. Therapeutic HIV vaccines: prior setbacks, current advances, and future prospects. *Vaccine* (2014). doi:10.1016/j.vaccine.2014.06.066
21. García F, León A, Gatell JM, Plana M, Gallart T. Therapeutic vaccines against HIV infection. *Hum Vaccin Immunother* (2012) **8**(5):569–81. doi:10.4161/hv.19555
22. Vieillard V, Strominger JL, Debré P. NK cytotoxicity against CD4+ T cells during HIV-1 infection: a gp41 peptide induces the expression of an NKp44 ligand. *Proc Natl Acad Sci U S A* (2005) **102**(31):10981–6. doi:10.1073/pnas.0504315102
23. Vieillard V, Le Grand R, Dausset J, Debré P. A vaccine strategy against AIDS: an HIV gp41 peptide immunization prevents NKp44L expression and CD4+ T cell depletion in SHIV-infected macaques. *Proc Natl Acad Sci U S A* (2008) **105**(6):2100–4. doi:10.1073/pnas.0711629105
24. Pollard RB, Rockstroh JK, Pantaleo G, Asmuth DM, Peters B, Lazzarin A, et al. Safety and efficacy of the peptide-based therapeutic vaccine for HIV-1, Vacc-4x: a phase 2 randomised, double-blind, placebo-controlled trial. *Lancet Infect Dis* (2014) **14**(4):291–300. doi:10.1016/S1473-3099(13)70343-8
25. Boffito M, Fox J, Bowman C, Fisher M, Orkin C, Wilkins E, et al. Safety, immunogenicity and efficacy assessment of HIV immunotherapy in a multi-centre, double-blind, randomised, Placebo-controlled Phase Ib human trial. *Vaccine* (2013) **31**(48):5680–6. doi:10.1016/j.vaccine.2013.09.057
26. Vardas E, Stanescu I, Leinonen M, Ellefsen K, Pantaleo G, Valtavaara M, et al. Indicators of therapeutic effect in FIT-06, a Phase II trial of a DNA vaccine, GTU(®)-multi-HIVB, in untreated HIV-1 infected subjects. *Vaccine* (2012) **30**(27):4046–54. doi:10.1016/j.vaccine.2012.04.007
27. Rodriguez B, Asmuth DM, Matining RM, Spritzler J, Jacobson JM, Mailliard RB, et al. Safety, tolerability, and immunogenicity of repeated doses of dermavir, a candidate therapeutic HIV vaccine, in HIV-infected patients receiving combination antiretroviral therapy: results of the ACTG 5176 trial. *J Acquir Immune Defic Syndr* (2013) **64**(4):351–9. doi:10.1097/QAI.0b013e3182a99590
28. Beignon A-S, Mollier K, Liard C, Coutant F, Munier S, Rivière J, et al. Lentiviral vector-based prime/boost vaccination against AIDS: pilot study shows protection against Simian immunodeficiency virus SIVmac251 challenge in macaques. *J Virol* (2009) **83**(21):10963–74. doi:10.1128/JVI.01284-09
29. DeBenedette M, Tcherepanova I, Gamble A, Horatinovich J, Harris J, Routy J-P, et al. Immune function and viral load post AGS-004 administration to chronic HIV subjects undergoing STI. In: *Paper presented at: 21st Conference on Retroviruses and Opportunistic Infections (CROI)*; Mar 3–6; Boston, MA (2014). Abst 343, p. 259–60. Available from: http://croi2014.org/sites/default/files/uploads/CROI2014_Final_Abstracts.pdf
30. Ensoli B, Fiorelli V, Ensoli F, Lazzarin A, Visintini R, Narciso P, et al. The preventive phase I trial with the HIV-1 Tat-based vaccine. *Vaccine* (2009) **28**:371–8. doi:10.1016/j.vaccine.2009.10.038
31. Longo O, Tripiciano A, Fiorelli V, Bellino S, Scoglio A, Collacchi B, et al. Phase I therapeutic trial of the HIV-1 Tat protein and long term follow-up. *Vaccine* (2009) **27**:3306–12. doi:10.1016/j.vaccine.2009.01.090
32. Bellino S, Francavilla V, Longo O, Tripiciano A, Paniccia G, Arancio A, et al. Parallel conduction of the phase I preventive and therapeutic trials based on the Tat vaccine candidate. *Rev Recent Clin Trials* (2009) **4**:195–204. doi:10.2174/157488709789957529
33. Ensoli B, Bellino S, Tripiciano A, Longo O, Francavilla V, Marcotullio S, et al. Therapeutic immunization with HIV-1 Tat reduces immune activation and loss of regulatory T-cells and improves immune function in subjects on HAART. *PLoS One* (2010) **5**:e13540. doi:10.1371/journal.pone.0013540
34. Van Regenmortel M, Andrieu JM, Dimitrov DS, Ensoli B, Hioe CE, Moog C. Paradigm changes and the future of HIV vaccine research: a summary of a workshop held in Baltimore on 20 November 2013. *J AIDS Clin Res* (2014) **5**:281. doi:10.4172/2155-6113.1000281
35. Watkins JD, Lancelot S, Campbell GR, Esquieu D, de Mareuil J, Opi S, et al. Reservoir cells no longer detectable after a heterologous SHIV challenge with the synthetic HIV-1 Tat Oyi vaccine. *Retrovirology* (2006) **3**:8. doi:10.1186/1742-4690-3-8
36. Tebas P, Stein D, Tang WW, Frank I, Wang SQ, Lee G, et al. Gene editing of CCR5 in autologous CD4 T cells of persons infected with HIV. *N Engl J Med* (2014) **370**(10):901–10. doi:10.1056/NEJMoa1300662
37. Kay MA, Walker BD. Engineering cellular resistance to HIV. *N Engl J Med* (2014) **370**(10):968–9. doi:10.1056/NEJMe1400593
38. Ye L, Wang J, Beyer AI, Teque F, Cradick TJ, Qi Z, et al. Seamless modification of wild-type induced pluripotent stem cells to the natural CCR5δ32 mutation confers resistance to HIV infection. *Proc Natl Acad Sci U S A* (2014) **111**(26):9591–6. doi:10.1073/pnas.1407473111
39. Didigu CA, Wilen CB, Wang J, Duong J, Secreto AJ, Danet-Desnoyers GA, et al. Simultaneous zinc-finger nuclease editing of the HIV coreceptors ccr5 and cxcr4 protects CD4+ T cells from HIV-1 infection. *Blood* (2014) **123**(1):61–9. doi:10.1182/blood-2013-08-521229
40. Didigu C, Doms R. Gene therapy targeting HIV entry. *Viruses* (2014) **6**(3):1395–409. doi:10.3390/v6031395
41. Esparza J. A brief history of the global effort to develop a preventive HIV vaccine. *Vaccine* (2013) **31**(35):3502–18. doi:10.1016/j.vaccine.2013.05.018
42. Lema D, Garcia A, De Sanctis JB. HIV vaccines: a brief overview. *Scand J Immunol* (2014) **80**(1):1–11. doi:10.1111/sji.12184
43. Gilbert PB, Ackers ML, Berman PW, Francis DP, Popovic V, Hu DJ, et al. HIV-1 virologic and immunologic progression and initiation of antiretroviral therapy among HIV-1-infected subjects in a trial of the efficacy of recombinant glycoprotein 120 vaccine. *J Infect Dis* (2005) **192**(6):974–83. doi:10.1086/432734
44. Pitisuttithum P, Gilbert P, Gurwith M, Heyward W, Martin M, van Griensven F, et al. Randomized, double-blind, placebo-controlled efficacy trial of a bivalent recombinant glycoprotein 120 HIV-1 vaccine among injection drug users in Bangkok, Thailand. *J Infect Dis* (2006) **194**(12):1661–71. doi:10.1086/508748
45. Buchbinder SP, Mehrotra DV, Duerr A, Fitzgerald DW, Mogg R, Li D, et al. Efficacy assessment of a cell-mediated immunity HIV-1 vaccine (the Step Study): a double-blind, randomised, placebo-controlled, test-of-concept trial. *Lancet* (2008) **372**(9653):1881–93. doi:10.1016/S0140-6736(08)61591-3
46. Johnson PR, Schnepp BC, Zhang J, Connell MJ, Greene SM, Yuste E, et al. Vector-mediated gene transfer engenders long-lived neutralizing activity and protection against SIV infection in monkeys. *Nat Med* (2009) **15**(8):901–6. doi:10.1038/nm.1967
47. Gray GE, Moodie Z, Metch B, Gilbert PB, Bekker LG, Churchyard G, et al. Recombinant adenovirus type 5 HIV gag/pol/nef vaccine in South Africa: unblinded, long-term follow-up of the phase 2b HVTN 503/Phambili study. *Lancet Infect Dis* (2014) **14**(5):388–96. doi:10.1016/S1473-3099(14)70020-9
48. Fauci AS, Marovich MA, Dieffenbach CW, Hunter E, Buchbinder SP. Immunology. Immune activation with HIV vaccines. *Science* (2014) **344**(6179):49–51. doi:10.1126/science.1250672
49. Qureshi H, Ma Z-M, Huang Y, Hodge G, Thomas MA, Dipasquale J, et al. Low-dose penile SIVmac251 exposure of rhesus macaques infected with adenovirus type 5 (Ad5) and then immunized with a replication-defective Ad5-based SIV gag/pol/nef vaccine recapitulates the results of the phase IIb step trial of a similar HIV-1 vaccine. *J Virol* (2012) **86**(4):2239–50. doi:10.1128/JVI.06175-11
50. Rolland M, Tovanabutra S, deCamp AC, Frahm N, Gilbert PB, Sanders-Buell E, et al. Genetic impact of vaccination on breakthrough HIV-1 sequences from the STEP trial. *Nat Med* (2011) **17**(3):366–71. doi:10.1038/nm.2316
51. Hertz T, Ahmed H, Friedrich DP, Casimiro DR, Self SG, Corey L, et al. HIV-1 vaccine-induced T-cell responses cluster in epitope hotspots that differ

from those induced in natural infection with HIV-1. *PLoS Pathog* (2013) **9**(6):e1003404. doi:10.1371/journal.ppat.1003404
52. Hammer SM, Sobieszczyk ME, Janes H, Karuna ST, Mulligan MJ, Grove D, et al. Efficacy trial of a DNA/rAd5 HIV-1 preventive vaccine. *N Engl J Med* (2013) **369**(22):2083–92. doi:10.1056/NEJMoa1310566
53. Michael NL, Robb ML. Phambili: moving forward without the blindfold. *Lancet Infect Dis* (2014) **14**(5):361–2. doi:10.1016/S1473-3099(14)70029-5
54. Tomaras GD, Ferrari G, Shen X, Alam SM, Liao HX, Pollara J, et al. Vaccine-induced plasma IgA specific for the C1 region of the HIV-1 envelope blocks binding and effector function of IgG. *Proc Natl Acad Sci U S A* (2013) **110**(22):9019–24. doi:10.1073/pnas.1301456110
55. Rerks-Ngarm S, Pitisuttithum P, Nitayaphan S, Kaewkungwal J, Chiu J, Paris R, et al. Vaccination with ALVAC and AIDSVAX to prevent HIV-1 infection in Thailand. *N Engl J Med* (2009) **36**:2209–20. doi:10.1056/NEJMoa0908492
56. Pitisuttithum P, Excler J-L, Kim J. Beyond RV144 efficacy results: an update. *Procedia Vaccinol* (2013) **7**:49–56. doi:10.1016/j.provac.2013.06.010
57. Kovacs JM, Nkolola JP, Peng H, Cheung A, Perry J, Miller CA, et al. HIV-1 envelope trimer elicits more potent neutralizing antibody responses than monomeric gp120. *Proc Natl Acad Sci U S A* (2012) **109**(30):12111–6. doi:10.1073/pnas.1204533109
58. Blattner C, Lee JH, Sliepen K, Derking R, Falkowska E, de la Peña AT, et al. Structural delineation of a quaternary, cleavage-dependent epitope at the gp41-gp120 interface on intact HIV-1 Env trimers. *Immunity* (2014) **40**(5):669–80. doi:10.1016/j.immuni.2014.04.008
59. Hansen SG, Vieville C, Whizin N, Coyne-Johnson L, Siess DC, Drummond DD, et al. Effector memory T cell responses are associated with protection of rhesus monkeys from mucosal simian immunodeficiency virus challenge. *Nat Med* (2009) **15**(3):293–9. doi:10.1038/nm.1935
60. Hansen SG, Ford JC, Lewis MS, Ventura AB, Hughes CM, Coyne-Johnson L, et al. Profound early control of highly pathogenic SIV by an effector memory T-cell vaccine. *Nature* (2011) **473**(7348):523–7. doi:10.1038/nature10003
61. Hansen SG, Piatak M Jr., Ventura AB, Hughes CM, Gilbride RM, Ford JC, et al. Immune clearance of highly pathogenic SIV infection. *Nature* (2013) **502**(7469):100–4. doi:10.1038/nature12519
62. Hansen SG, Sacha JB, Hughes CM, Ford JC, Burwitz BJ, Scholz I, et al. Cytomegalovirus vectors violate CD8+ T cell epitope recognition paradigms. *Science* (2013) **340**(6135):1237874. doi:10.1126/science.1237874
63. McMichael AJ, Koff WC. Vaccines that stimulate T cell immunity to HIV-1: the next step. *Nat Immunol* (2014) **15**(4):319–22. doi:10.1038/ni.2844
64. Fischer W, Perkins S, Theiler J, Bhattacharya T, Yusim K, Funkhouser R, et al. Polyvalent vaccines for optimal coverage of potential T-cell epitopes in global HIV-1 variants. *Nat Med* (2007) **13**(1):100–6. doi:10.1038/nm1461
65. Létourneau S, Im E-J, Mashishi T, Brereton C, Bridgeman A, Yang H, et al. Design and pre-clinical evaluation of a universal HIV-1 vaccine. *PLoS One* (2007) **2**(10):e984. doi:10.1371/journal.pone.0000984
66. Stephenson KE, SanMiguel A, Simmons NL, Smith K, Lewis MG, Szinger JJ, et al. Full-length HIV-1 immunogens induce greater magnitude and comparable breadth of T lymphocyte responses to conserved HIV-1 regions compared with conserved-region-only HIV-1 immunogens in rhesus monkeys. *J Virol* (2012) **86**(21):11434–40. doi:10.1128/JVI.01779-12
67. Barouch DH, Stephenson KE, Borducchi EN, Smith K, Stanley K, McNally AG, et al. Protective efficacy of a global HIV-1 mosaic vaccine against heterologous SHIV challenges in rhesus monkeys. *Cell* (2013) **155**(3):531–9. doi:10.1016/j.cell.2013.09.061
68. Monini P, Cafaro A, Srivastava IK, Moretti S, Sharma VA, Andreini C, et al. HIV-1 Tat promotes integrin-mediated HIV transmission to dendritic cells by binding Env spikes and competes neutralization by anti-HIV antibodies. *PLoS One* (2012) **7**(11):e48781. doi:10.1371/journal.pone.0048781
69. Cafaro A, Caputo A, Fracasso C, Maggiorella MT, Goletti D, Baroncelli S, et al. Control of SHIV-89.6P-infection of cynomolgus monkeys by HIV-1 Tat protein vaccine. *Nat Med* (1999) **5**:643–50. doi:10.1038/9488
70. Cafaro A, Titti F, Fracasso C, Maggiorella MT, Baroncelli S, Caputo A, et al. Vaccination with DNA containing *tat* coding sequences and unmethylated CpG motifs protects cynomolgus monkeys upon infection with simian/human immunodeficiency virus (SHIV89.6P). *Vaccine* (2001) **19**:2862–77. doi:10.1016/S0264-410X(01)00002-0
71. Maggiorella MT, Baroncelli S, Michelini Z, Fanales-Belasio E, Moretti S, Sernicola L, et al. Long-term protection against SHIV89.6P replication in HIV-1 Tat vaccinated cynomolgus monkeys. *Vaccine* (2004) **22**:3258–69. doi:10.1016/j.vaccine.2004.03.009
72. Borsetti A, Baroncelli S, Maggiorella MT, Moretti S, Fanales-Belasio E, Sernicola L, et al. Containment of infection in tat vaccinated monkeys after rechallenge with a higher dose of SHIV89.6P(cy243). *Viral Immunol* (2009) **22**:117–24. doi:10.1089/vim.2008.0082
73. Cafaro A, Bellino S, Titti F, Maggiorella MT, Sernicola L, Wiseman RW, et al. Impact of viral dose and major histocompatibility complex class IB haplotype on viral outcome in Tat-vaccinated mauritian cynomolgus monkeys upon challenge with SHIV89.6P. *J Virol* (2010) **84**:8953–8. doi:10.1128/JVI.00377-10
74. Demberg T, Florese RH, Heath MJ, Larsen K, Kalisz I, Kalyanaraman VS, et al. A replication-competent adenovirus-human immunodeficiency virus (Ad-HIV) tat and Ad-HIV env priming/Tat and envelope protein boosting regimen elicits enhanced protective efficacy against simian/human immunodeficiency virus SHIV89.6P challenge in rhesus macaques. *J Virol* (2007) **81**:3414–27. doi:10.1128/JVI.02453-06
75. Bachler BC, Humbert M, Palikuqi B, Siddappa NB, Lakhashe SK, Rasmussen RA, et al. Novel biopanning strategy to identify epitopes associated with vaccine protection. *J Virol* (2013) **87**:4403–16. doi:10.1128/JVI.02888-12
76. Krone WJ, Debouck C, Epstein LG, Heutink P, Meloen R, Goudsmit J. Natural antibodies to HIV-tat epitopes and expression of HIV-1 genes in vivo. *J Med Virol* (1988) **26**:261–70. doi:10.1002/jmv.1890260306
77. Demirhan I, Chandra A, Mueller F, Mueller H, Biberfeld P, Hasselmayer O, et al. Antibody spectrum against the viral transactivator protein in patients with human immunodeficiency virus type 1 infection and Kaposi's sarcoma. *J Hum Virol* (2000) **3**:137–43.
78. Binley JM, Klasse PJ, Cao Y, Jones I, Markowitz M, Ho DD. Differential regulation of the antibody responses to Gag and Env proteins of human immunodeficiency virus type 1. *J Virol* (1997) **71**(4):2799–809.
79. Reiss P, Lange JM, de Ronde A, de Wolf F, Dekker J, Debouck C, et al. Speed of progression to AIDS and degree of antibody response to accessory gene products of HIV-1. *J Med Virol* (1990) **30**:163–8. doi:10.1002/jmv.1890300303
80. Zagury JF, Sill A, Blattner W, Lachgar A, Le Buanec H, Richardson M, et al. Antibodies to the HIV-1 Tat protein correlated with nonprogression to AIDS: a rationale for the use of Tat toxoid as an HIV-1 vaccine. *J Hum Virol* (1998) **1**:282–92.
81. Re MC, Vignoli M, Furlini G, Gibellini D, Colangeli V, Vitone F, et al. Antibodies against full-length Tat protein and some low-molecular-weight Tat-peptides correlate with low or undetectable viral load in HIV-1 seropositive patients. *J Clin Virol* (2001) **21**:81–9. doi:10.1016/S1386-6532(00)00189-X
82. Richardson MW, Mirchandani J, Duong J, Grimaldo S, Kocieda V, Hendel H, et al. Antibodies to Tat and Vpr in the GRIV cohort: differential association with maintenance of long-term non-progression status in HIV-1 infection. *Biomed Pharmacother* (2003) **57**:4–14. doi:10.1016/S0753-3322(02)00327-X
83. Rezza G, Fiorelli V, Dorrucci M, Ciccozzi M, Tripiciano A, Scoglio A, et al. The presence of anti-Tat antibodies is predictive of long-term nonprogression to AIDS or severe immunodeficiency: findings in a cohort of HIV-1 seroconverters. *J Infect Dis* (2005) **191**:1321–4. doi:10.1086/428909
84. Bellino S, Tripiciano A, Picconi O, Francavilla V, Longo O, Sgadari C, et al. The presence of anti-Tat antibodies in HIV-infected individuals is associated with containment of CD4+ T-cell decay and viral load, and with delay of disease progression: results of a 3-year cohort study. *Retrovirology* (2014) **11**(1):49. doi:10.1186/1742-4690-11-4
85. Boily M-C, Baggaley RF, Wang L, Masse B, White RG, Hayes RJ, et al. Heterosexual risk of HIV-1 infection per sexual act: systematic review and meta-analysis of observational studies. *Lancet Infect Dis* (2009) **9**(2):118–29. doi:10.1016/S1473-3099(09)70021-0
86. Miyazawa M, Lopalco L, Mazzotta F, Lo Caputo S, Veas F, Clerici M, et al. The "immunologic advantage" of HIV-exposed seronegative individuals. *AIDS* (2009) **23**(2):161–75. doi:10.1097/QAD.0b013e3283196a80
87. Wu L, KewalRamani VN. Dendritic-cell interactions with HIV: infection and viral dissemination. *Nat Rev Immunol* (2006) **6**(11):859–68. doi:10.1038/nri1960
88. Hladik F, Sakchalathorn P, Ballweber L, Lentz G, Fialkow M, Eschenbach D, et al. Initial events in establishing vaginal entry and infection by human immunodeficiency virus type-1. *Immunity* (2007) **26**(2):257–70. doi:10.1016/j.immuni.2007.01.007
89. Laga M, Manoka A, Kivuvu M, Malele B, Tuliza M, Nzila N, et al. Non-ulcerative sexually transmitted diseases as risk factors for HIV-1 transmission in women:

results from a cohort study. *AIDS* (1993) **7**(1):95–102. doi:10.1097/00002030-199301000-00015
90. Mayer KH, Venkatesh KK. Interactions of HIV, other sexually transmitted diseases, and genital tract inflammation facilitating local pathogen transmission and acquisition. *Am J Reprod Immunol* (2011) **65**(3):308–16. doi:10.1111/j.1600-0897.2010.00942.x
91. Chun T-W, Murray D, Justement JS, Hallahan CW, Moir S, Kovacs C, et al. Relationship between residual plasma viremia and the size of HIV proviral DNA reservoirs in infected individuals receiving effective antiretroviral therapy. *J Infect Dis* (2011) **204**(1):135–8. doi:10.1093/infdis/jir208
92. Barouch DH, Santra S, Schmitz JE, Kuroda MJ, Fu TM, Wagner W, et al. Control of viremia and prevention of clinical AIDS in rhesus monkeys by cytokine-augmented DNA vaccination. *Science* (2000) **290**(5491):486–92. doi:10.1126/science.290.5491.486

Conflict of Interest Statement: The Guest Associate Editor Marc H. V. Van Regenmortel declares that, despite having collaborated with the author Barbara Ensoli, the review process was handled objectively and no conflict of interest exists. The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Received: 15 July 2014; accepted: 18 August 2014; published online: 08 September 2014.
*Citation: Ensoli B, Cafaro A, Monini P, Marcotullio S and Ensoli F (2014) Challenges in HIV vaccine research for treatment and prevention. Front. Immunol. **5**:417. doi: 10.3389/fimmu.2014.00417*
This article was submitted to HIV and AIDS, a section of the journal Frontiers in Immunology.

frontiers in
IMMUNOLOGY

OPINION ARTICLE
published: 10 June 2014
doi: 10.3389/fimmu.2014.00270

Epitopes for Protective Immunity Targeting Antigens of Pathogen and/or Host (EPITAPH): towards novel vaccines against HIV and other medically challenging infections

Salvador Eugenio C. Caoili*

Department of Biochemistry and Molecular Biology, College of Medicine, University of the Philippines Manila, Manila, Philippines
**Correspondence: badong@post.upm.edu.ph*

Edited by:
Marc H. V. Van Regenmortel, University of Strasbourg, France

Reviewed by:
Britta E. Wahren, Karolinska Institutet, Sweden
Dimiter S. Dimitrov, National Institutes of Health, USA
Lucia Lopalco, San Raffaele Scientific Institute, Italy

Keywords: epitopes, immunity, antibodies, pathogens, hosts, vaccines, HIV, AIDS

Antiretroviral therapy (ART) currently enables long-term survival of persons living with HIV (PLWH), but the respite from escalating pandemic AIDS-related deaths is undermined by both emergence of drug-resistant HIV and failure to develop HIV vaccines (1). Such reliance on drugs instead of vaccines is arguably a defeatist strategy against infectious diseases in the face of inevitable pathogen drug resistance. Still, the development of anti-infective vaccines is presently constrained by the exclusion of host self epitopes from candidate vaccine components. This aims to avoid inducing autoreactive host immune responses, some of which might yet be exploited to prevent or control infection by disrupting key host–pathogen interactions, notably using antibodies that bind critical epitopes and thereby sterically hinder molecular recognition (e.g., between virus and host cell). Classic vaccine-induced antibody responses are intended to target only pathogen-derived antigens or analogs thereof (e.g., recombinant or synthetic fragments); however, pathogen immune evasion may occur especially where epitope variability generates immunodominant decoy epitopes that elicit non-protective and possibly even harmful (e.g., infection-enhancing) antibody responses. Although vaccine design might be attempted to elicit protective antibody responses only against carefully selected pathogen epitopes, this is biologically unrealistic if based on a reductionist approach whereby individual epitopes are evaluated in isolation from one another, neglecting their functional interdependence in the context of host infection and immunity (2). Extending the notion of synergistic simultaneous targeting of structurally distinct epitopes, both pathogen-derived and host self epitopes are plausible targets of antibody-mediated protective immunity. As both pathogen and host contribute to pathogenesis of infectious disease (3), vaccination potentially can limit overall host damage due to both pathogen-associated (i.e., virulence) and host-associated (i.e., immune) factors, with some degree of host-induced damage being acceptable in place of more extensive pathogen-induced damage. An HIV vaccine thus might elicit antibodies that bind the gp120 receptor (CD4) or co-receptors (CCR4 and CCR5) to interrupt the viral replication cycle. This would be self-defeating if it resulted in excessive harm due to autoimmune host damage (e.g., manifest as quantitative or qualitative deficits of CD4+ cells, resulting in severe immunodeficiency), but tantalizing alternative scenarios are suggested by cases of natural resistance to HIV-1 infection that feature anti-CD4 autoantibodies (4).

Although binding of host self antigens by antibodies risks host damage, such binding may occur without resulting in appreciable harm. In support of this view, an apparent lack of pathological manifestations has been noted among healthy individuals who developed circulating antiplatelet autoantibodies (e.g., binding the platelet glycoprotein complex gpIb-IX) subsequent to immunization with recombinant HIV gp160 (5); and certain rare broadly neutralizing HIV-1 antibodies (e.g., to the 2F5 and 4E10 epitopes of HIV gp41) have been shown to exhibit polyspecific autoreactivity [e.g., such that the 2F5 and 4E10 antibodies bind the host phospholipid cardiolipin (6) yet also bind other human autoantigens including kynureninase and splicing factor 3b subunit 3, respectively (7)]. Such binding of host self antigens by autoantibodies entails broken self tolerance, which is typically difficult to induce (consistent with the rarity of 2F5- and 4E10-like antibodies, presumably reflecting an evolutionary adaptation that avoids autoimmune host damage); but in spite of this, immunization with recombinant constructs comprising CCR5-derived sequences recently has been shown (using murine and simian models) to elicit apparently non-deleterious anti-CCR5 antibody responses that block HIV (8) or SIV (9) infectivity, which points to the prospect of developing safe and efficacious HIV vaccines that induce protective immunity based on antibody targeting of judiciously selected host self epitopes (rather than whole autoantigens).

Hence, functional epitope mapping conceivably could delineate host self epitopes as targets for antibody binding *in vivo*, to block infection without producing excessive damage. Such targeting of host self epitopes might be sufficient to block infection (e.g., if binding of the epitopes by antibodies in itself imposed steric hindrance that directly precluded crucial biomolecular interactions); otherwise, infection still might be blocked by simultaneous binding of both host self- and pathogen-associated epitopes by

synergistically acting antibodies (e.g., with sufficient steric hindrance realized only between host- and pathogen-bound antibodies). Furthermore, host self epitopes tend to be highly conserved, which could facilitate vaccine design for entire host populations. The risk–benefit trade-off posed by host-reactive antibody-mediated immunity could be explored initially through passive immunization with monoclonal antibodies (mAbs), before committing to active-immunization strategies (e.g., that employ prophylactic or therapeutic vaccines). The mAbs could be developed in tandem with specific antidotes (e.g., anti-idiotypic constructs that prevent antibody binding of host self epitopes) for treating any adverse reactions that might occur, for instance, due to effector mechanisms such as complement activation and antibody-dependent cell-mediated cytotoxicity (ADCC). Antibody-mediated complement activation might be minimized by avoiding the juxtaposition of target epitopes (e.g., by targeting only one epitope per antigen), whereas damage due to any activated complement could be mitigated by complement-inactivating agents (e.g., eculizumab); and damage due to ADCC might be addressed by suppression of natural killer (NK) cell activity. Such complications could pose barriers to regulatory approval, albeit perhaps less so for therapeutic versus prophylactic vaccines particularly where net benefit (e.g., gained by obviating ART for PLWH) would be strongly compelling. Nevertheless, either or both complement activation and ADCC still might contribute to net benefit if they actually decreased pathogen replication [e.g., possibly with antibodies to the scavenger receptor CD36, which have been shown to inhibit HIV-1 release from infected macrophages by clustering newly formed virions at their site of budding (10)].

Beyond HIV, other medically challenging infections likewise might be controlled through antibody targeting of host self epitopes. In a manner analogous to the blocking of HIV infection by antibodies that bind CD4, CCR4, or CCR5, infection due to other pathogens can be blocked by antibodies and antibody-like constructs that bind appropriate target epitopes on host cells; this is exemplified by the anti-infective activity of multivalent recombinant antibody fusion proteins that bind intercellular adhesion molecule 1 (ICAM-1), particularly against human rhinovirus (for which ICAM-1 serves as the major host receptor) (11). Anti-infective immunity also may be mediated by antibodies that selectively target modified-self epitopes of infected host cells, such as band-3 neotopes of parasitized erythrocytes in falciparum malaria (12). Yet another strategy might be the use of anti-idiotypic antibodies against infection-enhancing antibodies, which is potentially applicable where antibody-dependent enhancement (ADE) of infection [e.g., with HIV, a wide variety of other viruses and even cellular pathogens including bacteria and protozoa (13)] contributes to pathogenesis, although caution would be warranted to avoid adverse iatrogenic effects (e.g., autoimmune damage mediated by antibodies produced against the anti-idiotypic antibodies, resulting from molecular mimicry of host self epitopes by the anti-idiotypic antibodies).

The scheme described herein thus shifts the focus of immunity-oriented approaches in health care, from immune destruction of specific targets back to the original object of vaccination, namely host protection against disease. This widens the scope of vaccines and immunotherapeutics, by placing due emphasis on immune targeting of host self epitopes as a potential means for host protection associated with negligible or justifiably limited host damage. More generally, immune targeting of epitopes may be broadly conceptualized in terms of high-level functional outcomes including both familiar consequences of conventional immunization regimens (e.g., for prophylaxis or therapy primarily based on targeted immune destruction of microbial pathogens and host-derived malignant cells) as well as less obvious and possibly even counter-intuitive but nonetheless beneficial results (e.g., host resistance to infections that is at least partly based on non-destructive binding of antibodies to host self epitopes). Such a perspective provides the basis for an expanded paradigm of biomedically enhanced immune function, the essence of which is concisely expressed as the idea of epitopes for protective immunity targeting antigens of pathogen and/or host (EPITAPH).

REFERENCES

1. Esparza J. A brief history of the global effort to develop a preventive HIV vaccine. *Vaccine* (2013) **31**(35):3502–18. doi:10.1016/j.vaccine.2013.05.018
2. Van Regenmortel MH. Basic research in HIV vaccinology is hampered by reductionist thinking. *Front Immunol* (2012) **3**:194. doi:10.3389/fimmu.2012.00194
3. Pirofski LA, Casadevall A. The damage-response framework of microbial pathogenesis and infectious diseases. *Adv Exp Med Biol* (2008) **635**:135–46. doi:10.1007/978-0-387-09550-9_11
4. Burastero SE, Figini M, Frigerio B, Lusso P, Mollica L, Lopalco L. Protective versus pathogenic anti-CD4 immunity: insights from the study of natural resistance to HIV infection. *J Transl Med* (2009) **7**:101. doi:10.1186/1479-5876-7-101
5. Kaplan C, Morel-Kopp MC, Kieny MP, Kolbe H, Salmon P, Sicard D, et al. Antiplatelet antibodies during the course of HIV-preventive vaccine trial. *AIDS* (1996) **10**(4):447–9. doi:10.1097/00002030-199604000-00019
6. Haynes BF, Fleming J, St Clair EW, Katinger H, Stiegler G, Kunert R, et al. Cardiolipin polyspecific autoreactivity in two broadly neutralizing HIV-1 antibodies. *Science* (2005) **308**(5730):1906–8. doi:10.1126/science.1111781
7. Yang G, Holl TM, Liu Y, Li Y, Lu X, Nicely NI, et al. Identification of autoantigens recognized by the 2F5 and 4E10 broadly neutralizing HIV-1 antibodies. *J Exp Med* (2013) **210**(2):241–56. doi:10.1084/jem.20121977
8. Pastori C, Diomede L, Venuti A, Fisher G, Jarvik J, Bomsel M, et al. Induction of HIV-blocking anti-CCR5 IgA in Peyer's patches without histopathological alterations. *J Virol* (2014) **88**(7):3623–35. doi:10.1128/JVI.03663-13
9. Van Rompay KK, Hunter Z, Jayashankar K, Peabody J, Montefiori D, LaBranche CC, et al. A vaccine against CCR5 protects a subset of macaques upon intravaginal challenge with simian immunodeficiency virus SIVmac251. *J Virol* (2014) **88**(4):2011–24. doi:10.1128/JVI.02447-13
10. Berre S, Gaudin R, de Alencar BC, Desdouits M, Chabaud M, Naffakh N, et al. CD36-specific antibodies block release of HIV-1 from infected primary macrophages and its transmission to T cells. *J Exp Med* (2013) **210**(12):2523–38. doi:10.1084/jem.20130566
11. Fang F, Yu M. Viral receptor blockage by multivalent recombinant antibody fusion proteins: inhibiting human rhinovirus (HRV) infection with CFY196. *J Antimicrob Chemother* (2004) **53**(1):23–5. doi:10.1093/jac/dkh019
12. Daniel-Ribeiro CT. Is there a role for autoimmunity in immune protection against malaria? *Mem Inst Oswaldo Cruz* (2000) **95**(2):199–207. doi:10.1590/S0074-02762000000200011
13. Halstead SB, Mahalingam S, Marovich MA, Ubol S, Mosser DM. Intrinsic antibody-dependent enhancement of microbial infection in macrophages: disease regulation by immune complexes. *Lancet Infect Dis* (2010) **10**(10):712–22. doi:10.1016/S1473-3099(10)70166-3

Conflict of Interest Statement: The author declares that the research was conducted in the absence of any

commercial or financial relationships that could be construed as a potential conflict of interest.

Received: 22 February 2014; accepted: 26 May 2014; published online: 10 June 2014.
*Citation: Caoili SEC (2014) Epitopes for Protective Immunity Targeting Antigens of Pathogen and/or Host (EPITAPH): towards novel vaccines against HIV and other medically challenging infections. Front. Immunol. **5**:270. doi: 10.3389/fimmu.2014.00270*
This article was submitted to HIV and AIDS, a section of the journal Frontiers in Immunology.

frontiers

EPFL Innovation Park · Building I · 1015 Lausanne · Switzerland
T +41 21 510 17 00 · info@frontiersin.org · frontiersin.org