Accelerating Next Generation Vaccine Development for Global Disease Prevention

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Summary

Vaccines are among the greatest successes in the history of public health. However, past strategies for vaccine development are unlikely to succeed in the future against major global diseases such as AIDS, TB, and malaria. For such diseases, the correlates of protection are poorly defined and the pathogens evade immune detection and/or exhibit extensive genetic variability. Recent advances have heralded in a new era of vaccine discovery. However, translation of these advances into vaccines remains impeded by lack of understanding of key vaccinology principles in humans. We review these advances towards vaccine discovery and suggest that for accelerating successful vaccine development, new human immunology-based clinical research initiatives be implemented with the goal of elucidating and more effectively inducing vaccine-induced protective immune responses.

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Introduction

At the end of the 18th century, Edward Jenner used cowpox-infected materials to immunize against smallpox and introduced the term " vaccine" (1). A century later, Louis Pasteur developed methods for attenuation of bacteria (2) and Salmon and Smith developed methods for inactivation of micro-organisms (3). Together, these advances ushered in a new scientific era of vaccinology. Virus propagation in cell culture enabled the development of methods for attenuating viral vaccines (4), leading to a golden age of vaccine development in the second half of the 20th century with the development of several vaccines including polio, measles, mumps, and rubella (5-9). By the latter part of the 20th century, most of the vaccines that could be developed by direct mimicry of natural infection with live attenuated or killed/inactivated vaccines had been developed. New technologies, including protein conjugation to capsular polysaccharides, and the advent of methods to engineer recombinant DNA, led to the development of vaccines for prevention of bacterial pneumonia and meningitis, hepatitis B and the recent development of the human papillomavirus vaccine (10-12). Vaccines have now led to the eradication of smallpox, near eradication of polio, prevention of untold millions of deaths from infectious diseases each year, and are one of the most effective public health measures available (13). For example, prior to the introduction of the measles vaccine in the United States, incidence of measles peaked at nearly 900,000 cases per year, compared with an average of less than 100 cases of measles per year in recent years in the US (14). Similarly, using metrics to measure cost-effectiveness of vaccines such as disability adjusted life year (DALY), global vaccination for measles results in $17 per DALY, one of the most cost-effective health interventions in developing countries (15). **Table 1** provides an overview of vaccine preventable diseases by currently licensed vaccines.

There are several diseases, however, that cause significant global morbidity and mortality, for which vaccines do not currently exist (**Table 2**). In general, the viruses, bacteria, and parasites for which new vaccines are needed, are either much more complex in their pathogenesis, exhibit extensive variability, or have evolved immune evasion mechanisms to thwart the human immune system. For example, there are many cases such as influenza and dengue viruses for which immunologic memory induced by natural infection protects against reinfection by homologous serotypes but not by heterologous serotypes (16). Thus, minor changes in the outer glycoproteins from circulating strains of the influenza virus result in the need for annual immunizations against influenza. For viruses such as respiratory syncytial virus (RSV), reinfection with the same virus can occur, though disease is generally less severe with these sequential re-infections (17). For HIV, the hyper-variability of the virus coupled with its capacity to integrate in the host genome, results in the inability of the host to clear the infection (18). Lastly, for pathogens such as cytomegalovirus (CMV), herpes simplex and Mycobacterium tuberculosis, a carrier state is established with reactivation occurring in situations of immunosuppression (19). Clearly, new vaccine discovery and novel immunization paradigms will likely be required for successful vaccine development against HIV, Mycobacterium tuberculosis, Plasmodium falciparum, hepatitis C (HCV), and other challenging pathogens for which there currently are no licensed vaccines.

Recent technological advances in molecular genetics, molecular and cellular immunology, structural biology, bioinformatics, computational biology, nanotechnology, formulation technologies and systems biology have heralded in a new era in immunogen design, adjuvant discovery (i.e. agents that enhance immune responses, and immune monitoring). However, translation of these advances into successful vaccines remains significantly impeded by a lack of understanding of key vaccinology principles in humans. This includes the need for greater understanding of disease-specific mechanisms of protective immunity, immune evasion mechanisms, and strategies to drive the immune system towards preferred responses by immunization.

Though based on sound scientific principles, currently licensed vaccines have largely been developed empirically and protection by these vaccines is generally conferred by antigen-specific antibodies, which prevent or reduce infection (20). Viral neutralizing antibodies prevent virus replication by blocking virus binding and entry into cells. For hyper-variable viral pathogens such as HIV and hepatitis C, it is likely that broadly neutralizing antibodies (bnAbs), (i.e. those targeting highly conserved regions of these viruses), will be necessary for globally effective vaccines. Many bnAbs against HIV have now been identified from HIV+ subjects (21), but most of these bnAbs exhibit a high level of somatic hypermutation (22). Moreover, there is currently limited understanding of the optimal immunization strategies in humans necessary to drive the antibody maturation process from germline to a mature broadly neutralizing antibody (23). Strategies for focusing immune responses on protective epitopes and away from immunodominant decoy epitopes are also undefined, as are the strategies for induction of long-term memory responses. Moreover, most of what we know of human immunology has come from the study of blood, whereas the battle between pathogen and the immune system largely takes place in lymph nodes and other tissues. Finally, little is known of ways to overcome neonatal immaturity and immune senescence in the elderly, important for the development and optimal coverage of next generation vaccines. While small animal and nonhuman primate models serve a critical role in basic science and preclinical vaccine discovery, they are significantly limited when it comes to extrapolation in humans, in large part due to differences in immunogenetics, species-specificity of pathogens, and the resident microbiota that influences species-specific responses.

In this review, we discuss the technological advances that are fueling a new era in vaccine discovery, highlight advances in immune monitoring technologies and systems biology that offer significant potential for discovering new biomarkers of protective immunity and identify the limitations of animal models for screening and prioritizing human vaccines. Based on this analysis, we conclude that new human immunology-based clinical research initiatives with the goal of elucidating and more effectively inducing vaccine-induced protective immune responses, would greatly accelerate the development of next generation vaccines against major global killers such as AIDS, TB and malaria and provide a foundation for vaccine development against new and emerging diseases.

The 21st Century Technological Revolution Fueling a New Era in Vaccine Discovery

Effective vaccines work by eliciting effector and memory immune responses that confer protection against infection and disease. New technologies have recently been developed that are now fueling a revolution in vaccine discovery, which should help to address vaccine development challenges such as pathogen diversity and immune evasion. These technologies can be divided into three major categories related to antigen discovery, adjuvants and vaccine delivery, and deciphering human immune responses.

Antigen Discovery Technologies

The capacity to sequence whole genomes of microorganisms and utilize bioinformatics for design of vaccines is a relatively recent approach to antigen discovery and has been termed reverse vaccinology (24). The first pathogen for which reverse vaccinology was attempted was Meningococcus B, the cause of 50% of global meningococcal meningitis. With the genome of the bacterium sequenced, over 600 potential antigens were assessed for antigenicity, resulting in the identification of over 90 newly detected surface proteins, 30% of which could induce bactericidal antibodies. A subset of these antigens, when formulated as immunogens induced protective immunity in mice, provides proof-of-concept for ongoing clinical development (25). Reverse vaccinology has now been applied to many other bacterial pathogens as full genome sequencing has advanced, including group B streptococcus, group A streptococcus, Streptococcus pneumoniae, Staphylococcus aureus, and Chlamydia (26). Besides reverse vaccinology, genomic-based antigen discovery has been enhanced by new technologies enabling interrogation of the comprehensive antigenic repertoire using libraries of genetically expressed antigens and screening for immunogenicity of the proteins during infection, termed " antigenome analysis" (27). In addition, advances in mass spectrometry have enabled direct testing of the presence and quantity of antigens on the surface of bacteria (28).

While these reverse vaccinology and antigenome technologies hold significant promise, particularly for identifying potential antigens for inclusion in vaccines, they remain limited in their capacity to predict which antigens are protective, as recently demonstrated by a Phase III trial of an experimental Staphylococcus aureus vaccine (29). Thus, greater efforts on understanding correlates of protection from human natural history studies, coupled with small, iterative clinical trials aimed at driving immune responses towards less immune dominant but protective epitopes, offers potential for greater utilization of these antigen discovery technologies.

Antigen discovery technologies have also advanced for identification of antigens to be used in vaccine candidates in order to induce neutralizing antibody responses, and for the generation of T cell-based vaccines. For pathogens which are hypervariable such as HIV "reverse engineering of vaccines", the concept that protective antigens could be identified through interrogation of the antibody repertoire from subjects infected with the pathogen (30) and formulated into effective vaccines is now being more fully exploited, due to recent technological advances in isolating monoclonal antibodies from memory B cells and plasma

cells from infected patients, deep sequencing and bioinformatics. These efforts include: 1) Identification of subjects with broadly neutralizing antibody serum responses (21); 2) Identification of broadly neutralizing monoclonal antibodies (bnAbs) from such subjects by single-cell memory B cell techniques with or without antigen selection, and cloning the heavy and light chains into IgG vectors (31-35); 3) Determination of the structure of the binding sites of such bnAbs at the structural level using crystallographic methods (31-35); and, 4) Mimicking the epitopic binding sites of such bnAbs on carrier protein scaffolds or vectors to serve as the basis for immunogens to elicit such bnAbs (32, 33) (**Figure 1)**. In addition to HIV, bnAbs against HCV (36) and influenza (37) are serving as templates for antigen designs for HCV and universal flu vaccines. The first proof-of-concept for reverse engineering of vaccines has now been achieved for respiratory syncytial virus (RSV), where computationally designed immunogens mimicking the binding site for an RSV neutralizing monoclonal antibody have successfully elicited RSV specific neutralizing antibodies in monkeys (38) .

The technologies above highlight advances in vaccine discovery for which antibodies to the pathogen are the principal biomarker of vaccine efficacy. Similarly, there have been significant technological advances in the past decade with respect to antigen discovery and evaluation of cell mediated immune (CMI) responses. CMI responses play an integral role in controlling many acute and chronic diseases, provide critical helper signals for elicitation of antibody responses, and thus are important components of vaccine development strategies against some parasites, intracellular bacteria and some viral infections. Recent technological advances in CMI assays including ELISPOT and Intracellular Cytokine Staining (ICS), mass spectrometry coupled with epitope identification algorithms (39), tetrameric staining reagents (40), genomics and proteomics of more complex pathogens such as mycobacterium and plasmodium (41, 42), and advances in immune monitoring (*see below*) have combined to enable significant advances for T cell-based vaccine antigen discovery. For example, long peptides encompassing conserved regions of HIV have been identified which induce polyfunctional T cells in macaques with breadth superior to single gene vaccines (43). In addition, computational optimization methods have been applied to the design of "mosaic" proteins, assembled from fragments of natural viral genetic sequences, that provide diversity coverage comparable to that of thousands of separate peptides but are tractable for vaccines (44).

These technologies are beginning to be applied both for antigen identification and for assessment of vaccine efficacy. With respect to antigen identification, epitope specific algorithms aimed at identifying key cellular immunity epitopes have been assessed for vaccinia virus, to better understand the protective components of the efficacious smallpox vaccine. Antigens recognized by CD4+ responses differed significantly from those recognized by CD8+ responses, with such data now being applied to reverse vaccinology strategies for development of epitope specific vaccines (45, 46) .

Simian immunodeficiency virus (SIV) vaccine efficacy studies with heterologous adenovirus vectors compared in nonhuman primates the level of Gag-specific CMI responses with viral load. Greater control of infection correlated with numbers of Gag-specific epitope responses (47). More recently, using MHC typed monkeys to probe the

efficacy of an SIV epitope-based vaccine, it was demonstrated that CMI responses to epitope-specific targets could control SIV infection in nonhuman primates (48). In contrast, studies with malaria immunogens demonstrated that heterologous vectors (chimp Adeno 63 + MVA) induced robust cellular immunity to specific blood stage antigens as measured by the magnitude of IFN-gamma ELISPOT responses, but had no effect on parasite growth rates in the blood (49) Similarly, a recently completed efficacy trial of MVA85A, a new tuberculosis vaccine in infants previously vaccinated with BCG, elicited modest CD4+ cellular immune responses as measured by ELISPOT and ICS assays, but failed to demonstrate efficacy against tuberculosis (50).

Adjuvants and Vaccine Vector Delivery Technologies

In the past decade, there have been significant advances in identification of signaling pathways and receptors of the innate immune system and a greater appreciation of the importance of innate immunity in influencing adaptive immune responses (51). Detection of microbes by the innate immune system is largely driven by pattern recognition receptors such as toll-like receptors (TLRs), that recognize common molecular structures found on microbes. In recent years, there has been a revolution in understanding of the receptors and molecules that drive innate immune responses (52-54), and this is now leading to the development and testing of novel vaccine adjuvants.

Adjuvants are an important component of vaccine formulations that potentiate immune responses via interaction with one or more TLRs, particularly important for those vaccines composed of proteins that are not highly immunogenic. For example, co-delivery of vaccine antigens with pattern recognition receptor agonists, may lead to enhanced immune responses to vaccines (55-57). This enables more mechanistic-based design of adjuvants, for potentiation of humoral and cellular immune responses. By extension, this should help to facilitate development of next generation adjuvants to help address vaccine challenges where immune responses may be compromised or less than optimal such as with immune senescence in the elderly. For example, adjuvant trials in individuals hyporesponive to hepatitis B vaccines showed that addition of CpG to the HBV vaccine enhanced the kinetics, magnitude and longevity of the seroprotective response (58). However, there is still a very limited database on improved efficacy in the elderly with adjuvanted vaccines, highlighting the need for additional studies in these populations. Adjuvants currently used in licensed vaccines include aluminum salts, oil in water emulsions, and virosomes and TLR4 agonists such as bacterially derived monophosphorylylipid A (MPL). In addition, there are a large number of adjuvants currently in development, aimed at boosting CD4+ helper T cell CD8+ cytotoxic T cell, and humoral immune responses (51) .

Many emerging and re-emerging pathogens, including those responsible for respiratory, gastrointestinal, and sexually transmitted diseases for which vaccines do not currently exist, initiate infection at a mucosal surface, suggesting that induction of mucosal immunity may be necessary or beneficial for prevention and control (59). Vector delivery systems designed to stimulate mucosal and systemic immunity have advanced in recent years, with some already in clinical trials including adenovirus, paramyxovirus, and some bacterial vectors (60). Viral vectors enable heterologous antigens to be delivered to antigen-processing

pathways needed to stimulate Human Leukocyte Antigen (HLA)-class I restricted cytotoxic T cell responses, in addition to priming for effective humoral immune responses. Further, replicating viral vectors more closely mimic the attributes of efficacious live attenuated vaccines (61). However, one of the major challenges for vector delivery systems is the need to overcome pre-existing immunity specific to the vector. For example, adenovirus type 5 (Ad5) is a potent vector for induction of cell mediated immune responses, yet the majority of the world's population has been previously exposed to Ad5 or closely related adenoviruses thus limiting the potential of this vector for vaccine development, particularly for use in the developing world. Strategies to overcome pre-existing or nascent anti-vector immunity are now being assessed, including use of vectors against which humans have low seroprevalence, simian and canine vectors for which pre-existing immunity in human populations is negligible, prime-boost regimens and administration of vector-based vaccines via a mucosal route. Low seroprevalence adenoviruses, such as Ad26, Ad35 and chimpAd63, are currently in clinical trials and preclinical studies with cytomegalovirus vectors have demonstrated the capacity for overcoming anti-vector immunity (62).

Dendritic cells (DC) are antigen presenting cells that provide initial surveillance mechanisms for exposure to pathogens, whereupon they undergo rapid maturation, and migrate to lymph nodes where induction of immune responses occurs. DCs play a central role in the induction of vaccine mediated immune responses, by providing antigen-specific and co-stimulatory signals required for T cell activation. Increased understanding of the mechanisms of antigen presentation, guided by elucidation of dendritic cell subsets and their functional plasticity, offer additional opportunities for targeting and potentiation of immune responses by vaccines (63). Targeting dendritic cells in vivo via antibodies to specific DC cell surface receptors, is now being explored for vaccines (64). Some viral vectors, such as vesicular stomatitis virus (VSV), target dendritic cells, which may account for the potent immune responses when VSV is used as a vector (65).

Deciphering Human Immune Responses

Along with advances in antigen discovery, adjuvant and vaccine vector delivery technologies, the past decade has seen a technological revolution in the capacity to analyze immune responses at the both the single cell and systems level, which offers tremendous potential for deciphering human immune responses to vaccines and identification of immune correlates of protection. Advances in systems biology, coupled with recent gains in understanding the pivotal role of innate immunity in augmenting adaptive immune responses, are now being applied to vaccine discovery in what is termed "systems vaccinology" (66). Technological advances with DNA microarrays and high through-put DNA sequencing, mass spectrometry powered proteomics, bioinformatics and computational methods enable data integration that serves as the basis of systems vaccinology (67). Initial studies on yellow fever vaccination in humans have identified early innate signatures that correlate with immunogenicity of the vaccines (68), and similar approaches are now being undertaken for influenza and malaria vaccine development. Interrogation of the antibody repertoire by deep sequencing and bioinformatics, has led to the identification of intermediates on the path to broadly neutralizing antibodies that is now helping to guide HIV vaccine development (69) (**Figure 2**). Concomitant with advances in

systems biology approaches, immune monitoring technologies have advanced to enable new approaches to characterize and interrogate human immune responses (70). Multi-plexed flow cytometric and intracellular cytokine staining assays now allow analyses of cell phenotype, subset identity, activation and intracellular signaling status and effector activities (71-73), and single-cell gene expression analyses allow greater specificity in immune monitoring. Such monitoring should enable greater precision in assessments of correlates of protection for currently licensed vaccines, which will help guide next generation vaccine development. For example, induction of effector memory responses as determined by flow cytometric analysis is thought now to play an important role in the significant control of SIV infection conferred by CMV vector-based vaccine candidates (62). Combining these tools with other new methods for analysis of antigen-specific cells using multiplexed tetramer technologies, and highly sensitive microarray and sequencing technologies, will allow not only for assessment of responses in blood, but due to the sensitivity of these methods, may also provide insights into selected tissue level responses. This will enable future analyses to be made in the tissues, where the battle between pathogen and host largely takes place, rather than simply relying on measurements from peripheral blood.

Taken together, recent progress in technological development for antigen discovery, adjuvant and vector discovery and technologies aimed at deciphering human immune responses, provide the foundation for significant advances in vaccine discovery and their application towards accelerating vaccine development against the major global diseases for which vaccines do not currently exist.

The Importance of Studying Vaccines in Humans: Limitations of Current Animal Models

Despite best efforts, in vitro and small animal models do not effectively recapitulate the dynamics of human immune responses to vaccines. Mouse models, in particular the use of inbred mice, have been extremely effective as a tool for basic immunologists, yet have been largely unsuccessful as models for clinical application (74, 75). For example, inbred mice often have a number of homozygous recessive defects that alter the regulation of immune responses (76). Differences in pattern recognition receptors e.g. TLR9 expression, may account for significant differences between humans and rodents in response to microbial stimuli (77, 78). In addition, protocols in small animal models do not adequately reflect human vaccination studies. Many murine studies utilize intravenous or intraperitoneal injections, whereas human vaccines are generally administered intramuscularly or subcutaneously (79). Different routes of immunization, for example mucosal vs. intramuscular, can change patterns of recognition by dendritic cell subsets, leading to modifications in immune responses (80). In addition, dose and regimen of immunizations are often different between small animal studies and human clinical trials, which can affect the quality and quantity of priming, effector and memory responses.

As a result of these deficiencies, there is renewed interest in the use of "humanized" mice, which are usually immunodeficient mice that are reconstituted with human hematopoietic stem cells, peripheral blood mononuclear cells or tissue transplants. Although these models are potentially very promising, they have yet to be validated for predicting human immune

responses to licensed vaccines, and human hematopoietic cells developed from stem cell transplantation in immunodeficient mice are not always phenotypically and functionally identical to those that develop in humans (81). Thus, such mice may not fully recapitulate human immune responses, particularly where T cell helper and other effector cell mechanisms are integral for optimization of vaccine-induced immune responses.

Although nonhuman primates have played an important role in the development of hepatitis B and other vaccines, there are additional limitations of nonhuman primate models with respect to human vaccine development, including differences in immunogenetics between macaques and humans, species specificity of some viral vectors being developed as vaccine candidates, and the impact of the microbiome of humans on vaccine evaluation. For example, HIV vaccine developers primarily rely on simian-immunodeficiency virus (SIV) replication in rhesus macaques, as a challenge model for assessing vaccine concepts, due to the limited replication of HIV in macaques (82). However, the hyper variability of HIV cannot readily be modeled by SIV, due to the limited numbers of referenced strains of SIV. Moreover, differences between major histocompatibility complex (MHC) restrictions in monkeys and HLA restrictions in human='s limits the assessment of epitopes for inclusion in vaccine candidates. In addition, vectors carrying HIV genes such as cytomegalovirus and replication competent Adenovirus type 4 are species-specific for humans.

The microbiome of humans consists of the plethora of viruses, bacteria, and parasites that infect and reside in our tissues, contributing a significant proportion of genetic information to our metagenome, and thus affecting susceptibility and resistance to disease, particularly inflammatory diseases such as type 1 diabetes, ulcerative colitis and Crohn's disease (83). The concept of microbiome is being applied to personalized medicine, but also should be viewed as a limitation when contemplating animal models for human vaccine development (84).

In summary, although murine models have provided important insights into basic immunology, and small animal models plus nonhuman primates have been important in the development of some currently licensed vaccines, there are significant limitations of these models in predicting human responses to vaccines. This was demonstrated yet again when SHIV protection studies in monkeys suggested that an Ad5-based HIV vaccine could suppress viral load and control disease, yet human efficacy trials failed to confirm these observations (85, 86). Thus, greater attention needs to be focused on immunogenicity studies in humans aimed at answering specific questions that impede vaccine development, and translating this information towards the development of next generation and more efficacious vaccines against globally important diseases.

Future Directions: Optimizing Protective Immune Responses in Humans

Although licensed vaccines continue to provide tremendous public health benefits, success rates in vaccine development are not optimal, and are even worse for the subset of complex pathogens for which viability and immune evasion mechanisms present additional challenges. For example, in one survey of over 200 vaccine development projects, success rates for vaccines was only 22% compared with 40 % for biopharmaceuticals (87). In our

view, this high rate of failure for vaccines is directly related to: 1) Lack of information about mechanisms for protective immunity directly applicable to pathogen-specific vaccine development; and, 2) Lack of understanding of optimal strategies to elicit vaccine-induced protective immune responses in humans and thus inability to effectively predict which immunogens would have a greater chance for success in vaccine efficacy trials.

For the major global diseases for which vaccines do not currently exist (**Table 2**), identification of vaccine-induced protective immune responses will require greater understanding of pathogen-specific mechanism(s) of protective immunity, particularly from human natural history studies and in some cases from human challenge models when available, such as malaria. This is important in cases where natural infection by specific pathogens provide protection against subsequent exposure but also in cases where natural infection does not confer such protection. Thus, greater linkages of human disease-specific pathogenesis studies with applied vaccinology studies, will be central to accelerating next generation vaccine development.

Moreover, when one factors in the current lack of understanding in humans of how to fine control the antibody affinity maturation that is likely required to elicit broadly protective responses to highly antigenically variable pathogens; of how to focus immune responses on subdominant yet critical protective epitopes; and of how to elicit long-term central and effector memory responses, it's no surprise that vaccine success rates against complex pathogens such as HIV, mycobacteria, and plasmodium are worse than those against non-variable, acute infections. However, the confluence of recent technological advances for vaccine discovery, systems biology and immune monitoring, yields a tremendous opportunity for accelerating vaccine development. If harnessed effectively, they can lead to a greater understanding of disease-specific mechanisms of protective immunity, elucidate key principles of vaccinology and be used to optimize humoral and cellular protective immune responses. Application of this information across the spectrum of disease-specific vaccine development programs will likely shorten the timelines for successful development of new and improved vaccines.

Clinical trials of vaccines are currently driven by product-specific issues, as sponsors or vaccine developers have preferred to quickly advance candidates from Phase I/II safety immunogenicity trials, to Phase IIb/III efficacy trials, rather than prioritize vaccine discovery related issues. Unfortunately, for diseases such as HIV, TB, malaria and others, the gap in understanding how best to elicit the requisite humoral and cellular effector and memory responses in humans has slowed the pace of vaccine development. In the absence of a greater understanding of these key vaccinology principles in humans, the vaccine field is left to rely on large and expensive field trials to provide any guidance. Such trials can cost $50 million - $100 million or more, take several years to complete and often are undertaken with low probabilities of success. This is not to suggest that field trials should be abandoned. It is rather to suggest that in parallel, significant resources be directed towards conducting small, iterative, human clinical studies to address key questions currently impeding the development of next generation candidate vaccines.

In summary, successful development of vaccines against the major global diseases for which vaccines do not currently exist would be transformational for public health, with huge benefits across society. Historical paradigms of empirical product development efforts alone will unlikely be successful against major global killers that cause significant morbidity and mortality today. This is due in large part to the current lack of the understanding of mechanisms for protective immunity, immune evasion mechanisms, and immunization strategies on how best to elicit protective immune responses against such pathogens in humans to mimic the best attributes of successful licensed vaccines. To accelerate next generation vaccine development, we suggest that new human immunology-based clinical research initiatives be established, focused on addressing key questions impeding vaccine development, with the goal of elucidating and more effectively inducing vaccine-induced protective immune responses. Collectively, such a "Human Vaccines Project" holds the potential to greatly accelerate the development of next generation vaccines against major global killers such as AIDS, TB, malaria and other infectious diseases, enable more successful vaccine development against allergies, autoimmune diseases, and cancers, and provide a foundation for vaccine development against new and emerging diseases.

Acknowledgments

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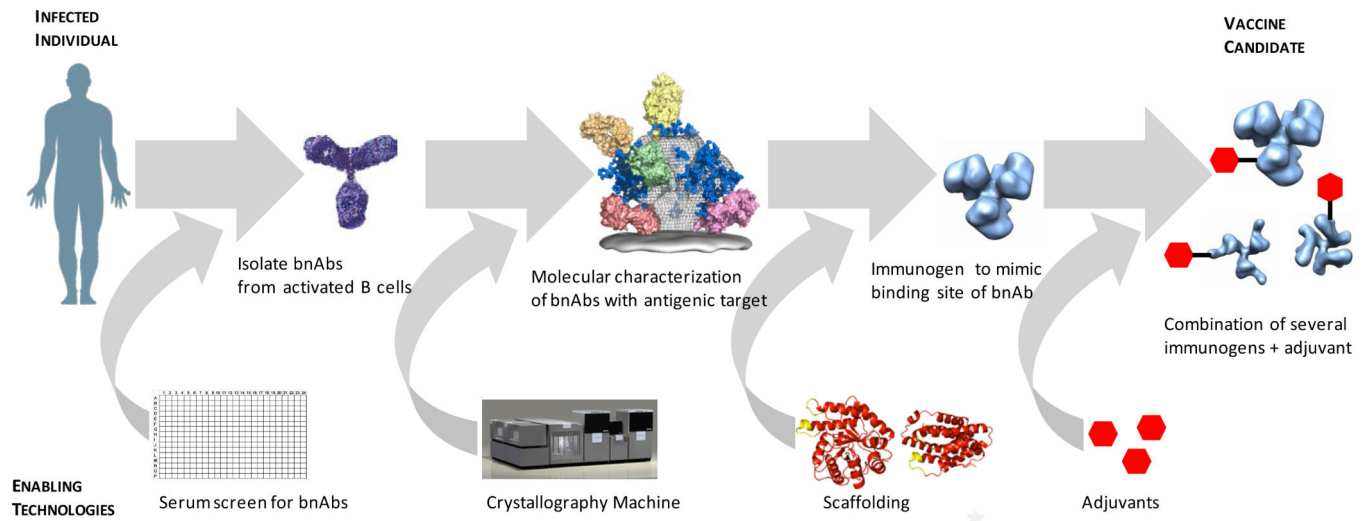
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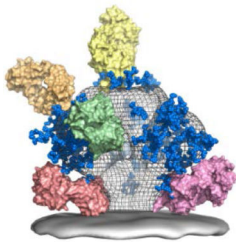38. Schief, W. AIDS Vaccine. Suppl 2. Vol. 9. Boston, Massachusetts, USA: 2012. 2012. p. S03.04

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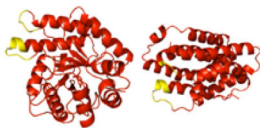
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INFECTED INDIVIDUAL

Isolate bnAbs from activated B cells

Molecular characterization of bnAbs with antigenic target

Immunogen to mimic binding site of bnAb

VACCINE CANDIDATE

Combination of several immunogens + adjuvant

ENABLING TECHNOLOGIES

Serum screen for bnAbs

Crystallography Machine

Scaffolding

Adjuvants

Source: Burton lab, TSRI, unpublished

Source:, Burton et al, Science 337:183, 2012

Source: Bill Schief lab, unpublished

Source: Burton lab, TSRI, unpublished

Figure 1. Reverse engineering of vaccines

The figure schematically depicts the identification of bnAbs; technological advances in high-throughput robotic crystallization platforms to enable greater precision in identifying the epitope targets for bnAbs; computational methods to design scaffolds mimicking the binding site of the bnAbs; and lastly greater understanding at the mechanistic level for selection of adjuvants to include in vaccine formulations for potentiating immune responses.
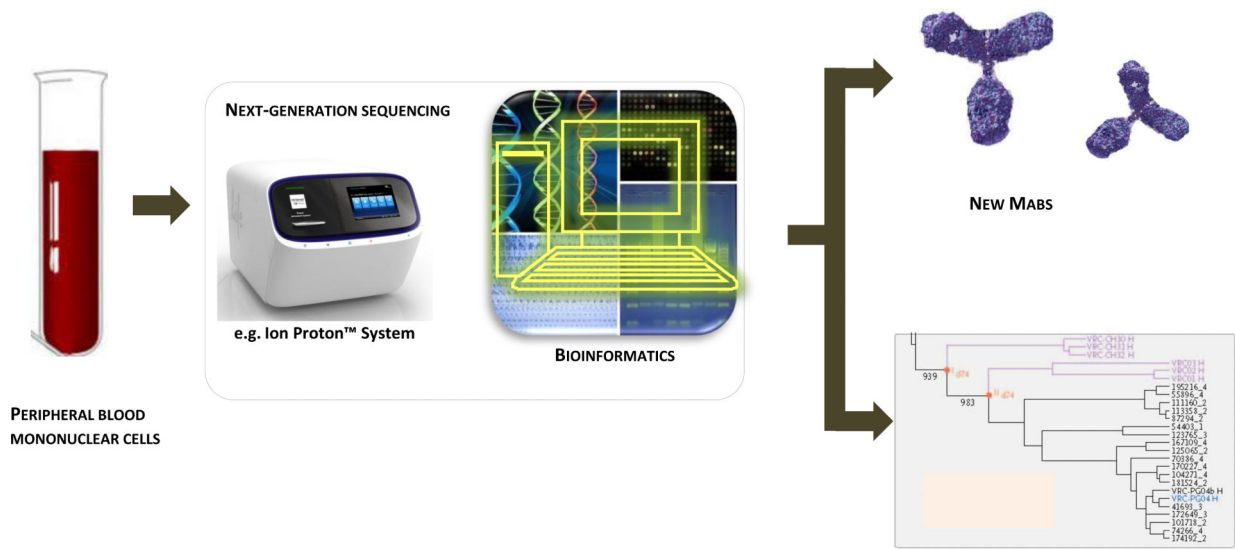
MATURATION PATHWAYS AND INTERMEDIATES

Source: Adapted from Nabel, G N ENGL J MED 368;551 (2013)

Figure 2. Technological advances towards deciphering human immune responses
This figure depicts technological advances in deep sequencing and bioinformatics which now allow identification of the antibodyome and tracing antibody development from germline through somatically hypermutated intermediates to the designated fully mature antibody Such tracing can help guide B cell lineage-based vaccine design (23).

Table 1

Major Global Infections Prevented by Vaccines[a]

Bacterial	Viral
Cholera	Adenovirus Based Diseases
Diphtheria	Hepatitis A
Haemophilus Influenza	Hepatitis B
Meningococcal Meningitis	Human Papillomavirus
Plague	Influenza
Pneumococcal Pneumonia	Japanese Encephalitis
Tetanus	Measles
Tuberculosis	Mumps
Typhoid Fever	Polio
	Rabies
	Rotavirus Diarrhea
	Rubella
	Smallpox
	Tick Borne Encephalitis
	Varicella-Zoster
	Yellow Fever

[a]The level of efficacy for the vaccines noted above ranges in different populations and regions of the world.

Table 2

Major Global Diseases for which Vaccines do not Currently Exist

Campylobacter
Chlamydia
Cytomegalovirus
Dengue
Epstein-Barr (Mononucleosis)
Helicobacter pylori- Gastrointestinal ulcers
Hepatitis C
Herpes Simplex
HIV
Influenza (Universal flu vaccine to replace need for annual flu vaccine)
Leishmaniasis
Malaria
Respiratory syncytial virus
Rhinovirus
Schistosomiasis
Shigella
Streptococcus Group A and B
Tuberculosis
Urinary tract infections
OTHER
Allergies; Autoimmune diseases; Cancers[b]

[b] HBV and HPV vaccines are effective in preventing liver and cervical cancers respectively