HIV, Tuberculosis, and Malaria

JAN WILHELM

SABIN
VACCINE INSTITUTE

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Jan Wilhelm, MD
Professor of Pediatrics, Clínica Alemana — Universidad del Desarrollo School of Medicine, Santiago de Chile
Member, Chilean Technical Advisory Group on Immunizations, Chilean Infectious Diseases Society Advisory Committee on Immunization Practices and Chilean Institute of Public Health Vaccines Pharmacovigilance Committee

Introduction

The acquired immunodeficiency syndrome (AIDS), tuberculosis (TB), and malaria, collectively, account for over 5 million deaths a year. However, because of their genetic instability, great variability or ability to hide within host cells, they have been able to avoid the conventional development of effective vaccines. Globally they represent one of the greatest challenges for public health in the second decade of the XXI century. Recent trials have evidenced the feasibility to develop vaccines that can prevent the infection caused by the human immunodeficiency virus (HIV) and malaria.

Furthermore, advances in vaccine development, including new adjuvants, new administration schedules, and strategies for the presentation of antigens at the intracellular level have led to progress in the development of a better tuberculosis vaccine. New tools, such as the so-called systems biology and vaccine design based on the structure of antigens, will hopefully deepen the understanding of the protection mechanisms which, in turn, will result in the development of vaccines against these pathologies.

Overview of HIV, TB, and Malaria

What do these three microorganisms have in common? They all pose a challenge for humanity. To date, HIV/AIDS has caused over 25 million deaths; 33 million people are currently living with HIV, and 2.6 million new cases emerge every year, resulting in 1.8 million deaths annually.[1,2] In the case of malaria, 225 million new cases and one million deaths occur every year.[3] Finally, tuberculosis impacts a third of the world population, results in 9.6 million new cases and 1.7 million deaths a year; treatment has been challenged by the emergence of multidrug-resistant tuberculosis.[4] Broad genetic heterogeneity and ability to hide at intracellular level are common among the three pathogens.

Structural Vaccinology and Systems Biology

However, new technological advances provide hope. New immunization schedules, new adjuvants, and new methods for antigen presentation are being tested. Moreover, innovative ways to treat these illnesses have resulted in the identification of adequate protection markers and clinical and regulatory innovations. Structural Vaccinology comprises the design of new antigens based on the already-known structure of surface proteins while exposing preserved epitopes or creating molecules with multiple immuno-dominant epitopes to induce better protective immune responses.[5] Systems Biology is a strategy to address biological problems by collecting and integrating data at various levels, thus revealing properties that cannot be demonstrated or predicted otherwise, such as for example the response to a new vaccine dependent on genetic, molecular, and environmental factors and their interaction.[6] By means of a computational analysis, models can be developed to forecast whether a vaccine will produce an adequate protective response or not.[7,8]

Current Status of HIV, TB, and Malaria Vaccines

HIV

Why don't we already have a vaccine against HIV? The absence of a vaccine has not been because of a lack of effort, rather the capacity HIV has to escape: immediate and final integration with the genome in the host cells, variability of the epitopes to which the antibodies and T cells bind, and weak neutralizing antibodies, as illustrated by the absence of a spontaneous cure or recovery from the HIV infection.

The genome sequence of the virus is highly variable. The world population of HIV viruses is divided into four main groups (A, B, C, and E) mainly present in Africa, North America and Europe, Asia, and Africa, respectively. Within each group, the sequence varies tremendously and the virus continues to evolve and mutate in every patient infected with the virus. Neutralizing antibodies against the virus and the T-cells induced by the natural infection or conventional vaccination generate a narrow immune response, which is inadequate to confer protection against all of the virus variants.

The HIV protective immunity lacks proper markers. Regarding the antibodies, no significant relationship exists between the neutralizing antibodies and the viral control; however, some response has been observed following the passive transfer of anti-HIV and anti-simian immunodeficiency virus (SIV) neutralizing monoclonal antibodies. As to specific anti-HIV CD8 T-cells, depletion of CD8 cells in rhesus monkeys results in the immune system losing control over SIV, the magnitude of the response being inversely proportional to the viral load (VL) in acute and chronic patients and in elite controllers. The quality of the lymphocytic response, as expressed in its multi-functionality, differentiation and avidity, is also important. HIV-specific CD4 T-helper-1 cells exhibit the inverse relationship to the viral load, both in the acute infection phase and in the dormant infection phase.

Scientists from all over the world have been attempting to develop an effective HIV vaccine for over two decades. In the nineties, Phase I and Phase II clinical studies were conducted with subunit vaccines from the HIV envelope. However, the neutralizing antibodies (Nabs) *in vitro* only neutralized the vaccine strains, which is unacceptable for HIV given the high-frequency at which the virus mutates surface antigens.[9, 10] Later on, recombinant subunit vaccines were tested but the results were negative due to the antigen diversity and the Nabs inability to neutralize wild strains.[11, 12] Studies geared to activate T-cell immunity against HIV, such as STEP or the RV144 study, attained 30% protection or lower.[13, 14]

Nowadays it is clear that neither the humoral protection nor the cellular protection by themselves will be sufficient to develop a protective vaccine. Unfortunately, combined strategies attained only marginal protection. Through systems biology, immunogenicity markers have been studied (CD4-specific, CD8-specific, viral load) to generate more stable gp120 and gp41 molecules, with preserved epitopes, which in turn has translated into broad-spectrum Nabs as the only strategy proven to prevent the HIV infection.[15]

The identification of immuno-dominant neutralizing epitopes from HIV variants will likely be the basis for the development of new membrane proteins for broader protection. Other supplementary strategies include non-neutralizing antibodies against preserved antigens to broaden immunity (due to mosaic antigens or preserved chimeric antigens), T-cell-based vaccines to control viruses that mutate due to the selective pressure of the neutralizing antibodies and new vectors.

Despite the significance and hope behind the progress made in recent years, all of the forecasts for the availability of an effective HIV vaccine have failed, which makes it impossible to speculate how far we are from the attainment of the goal.

Tuberculosis

TB is caused by the bacteria *Mycobacterium tuberculosis* that infects the lungs by penetrating them and growing inside the macrophages. The immune cells surround the infected macrophages and form granulomas where the bacteria may remain dormant for a while. The weakening of the immune system with the HIV infection paves the way for the reactivation and ensuing disease.

On an annual basis 9.6 million individuals will develop TB and 1.5 million will die. However, TB incidence has been diminishing by 1.5% every year since 2000 and mortality has diminished by 47% since 1990. The greatest problem is the emergence of multidrug resistant TB (MDRTB). Annually 3.3% new cases emerge, 20% relapse and almost 10% of MDRTB is extremely resistant or utterly impossible to treat. MDRTB poses a challenge for the development of new TB vaccines.

M. tuberculosis poses multiple difficulties for the development of an ideal vaccine. At the outset, the antigens are complex: different proteins on the cellular wall and inside, some secreted at various stages of the infection; glycoproteins; sugars; microlipids, and lipids (lipids do not present themselves as protein antigens traditionally do and there are no lipid vaccines [Koch removed the lipids from purified protein derivative (PPD)]). Furthermore, *M. tuberculosis* presents a complex vital cycle: log growth, multiple immuno-dominant antigens secreted at each stage, ability to stay in immuno-salient latency (latency genes, latency antigens) and subsequently reactivate as an active disease.

Do we need four different TB vaccines? The question is valid since non-immune patients present the primary disease; immune and sensitized patients experience the post-primary disease, the dormant disease, and the need to optimize treatment for the disease. Therefore, the immunization strategies proposed to control the disease are infection prevention (*prime*), disease prevention (*booster*) and prevention of relapses (therapeutic). The tuberculosis vaccine based on an attenuated *Mycobacterium bovis* strain, or bacille Calmette–Guerin (BCG), has been used for close to 100 years but its efficacy is controversial.[16, 17]

The BCG vaccine may prevent the spread of the disease and deaths in children but not chronic infection or pulmonary tuberculosis in adults. However, depending on the studies, efficacy ranges between 0% protection against any disease (study conducted in Madras/Chennai, India)[18] and up to 80% against miliary TB and meningeal TB in children (study conducted in the United Kingdom)[19] through 50% protection against pulmonary TB (study conducted in the United States).[20-23] Furthermore, currently there are several BCG strains but the BCG manufacturing techniques are not part of the existing production practices and we are unaware if the BCG vaccine generates a proper primary immune response against *M. tuberculosis*.[24]

At this time, 16 new vaccines are undergoing clinical trials (proof-of-concept or Phase IIb studies): with recombinant antigens, DNA or viral vectors and subunit vaccines as BCG booster (to prevent chronic infection or avoid reactivation).[25-27] However, the most advanced vaccine is the one that entails the reengineering of the BCG vaccine itself.[28]

Challenges for future studies of TB vaccines include geographical diversity in terms of risk of infection and TB disease, definition of clinical target (infection, disease, latency or cure, duration, level of acceptable efficacy, integration or replacement of BCG vaccine, prioritization of potential vaccines and impact of HIV epidemiology).

Malaria

Malaria is caused by the *Plasmodium* parasite, which infects humans through a mosquito bite. The mosquito injects the parasite in the form of a sporozoite that quickly migrates to the liver. Following 6 to 7 days it is released in a different form, as an amerozoite, infecting and multiplying inside red blood cells. Finally, a new form of the parasite (gametocyte) is generated in the human host and acquired once again through mosquito bites. *Plasmodium falciparum* and *Plasmodium vivax* are the main human pathogens. The various stages of the parasite have different antigen compositions; antigen variability within each stage has been one of the main obstacles to the development of a vaccine.

Natural immunity against malaria is specific to each stage of the disease, but naturally-acquired immunity develops slowly, and incompletely for a limited amount of time. In spite of recent advances in the reduction of malaria mortality due to other interventions (48% reduction since 2000), every minute a child in Africa dies due to malaria. Moreover, success is undermined by the financial instability of the affected countries and resistance to artemisinin and insecticide. Therefore, vaccines are urgently needed to reduce the incidence and deaths caused by the disease as well as to block transmission of the parasite through herd immunity and allow for the elimination and eradication of the disease.

The most advanced vaccine in clinical trials (RTS, S) completed a Phase III evaluation in African children from 13 centers, in eight countries. Over 12 months of follow-up, RTS, S demonstrated approximately 50% protection against the clinical disease caused by *Plasmodium falciparum* in children aged 5 to 17 months and about 30%

protection in children aged 6 and 12 weeks, when administered together with vaccines from the Immunization Program.[29] In spite of waning immunity [In participants aged 5–17 months, the half-life of the short-lived component of the antibody response was 45 days (95% credible interval 42–48) and that of the long-lived component was 591 days (557–632)], there is a clear benefit to the vaccine.

An average of 1,363 cases of clinical malaria are estimated to have been prevented over 4 years of follow-up per 1,000 vaccinated children and 1,774 cases are estimated to have been prevented amongst those who received the booster.

The World Health Organization (WHO) monitored a process to enable understanding of the differences in the epidemiological models developed by four different groups (Imperial College, Swiss TPH, Intellectual Ventures, GSK) intended to reach consensus on impact and cost-effectiveness. All the models forecast a 10% to 28% reduction in malaria-related mortality in children < 5 years who received the full schedule. In areas with moderate-to-high transmission, this translates into the prevention of 116,500 cases of clinical malaria and 484 deaths every 100,000 children vaccinated.

At a hypothetical price of US$ 5/dose, the average incremental cost-effectiveness rate of the vaccine is US$ 87 ($48-$244) per DALY prevented and US$ 25 ($16-$222) per clinical case prevented, which is favorable when compared to the global cost-effectiveness estimated for other vaccines. Based on a comparative cost-effectiveness study conducted by the Imperial College of London, long-lasting insecticide nets (LLINs) are the most cost-effective initial intervention in all of the scenarios, followed by seasonal malaria chemoprophylaxis where recommended and, lastly, RTS, S in places with parasite prevalence > 10%.

WHO recommended conducting pilot studies with RTS, S/AS01 in 3 to 5 sites with a high burden of disease in Africa. The studies should assess the operational feasibility of providing the vaccine to the target population under the four-dose schedule recommended within the context of the local health services, the impact of the vaccine on all-cause child mortality when implemented concomitantly with other interventions recommended against malaria and surveillance of adverse events following vaccination, in particular meningitis and cerebral malaria, before considering coverage escalation.

A drop in the disease caused by *P. falciparum* will prioritize the development of a vaccine against *P. vivax*. However, work is being conducted to improve human immunization models (issues with relapses and lack of *P. vivax* cultures). The first *P. vivax* trial used a recombinant *P. vivax* CS protein in AS01, but the clinical evaluation may be difficult given the potential interactions with *P. falciparum* and the differentiation of new infections from hypnozoite reactivation.

The development of more efficient vaccines to prevent the clinical disease caused both by *P. falciparum* and *P. vivax*, as well as vaccines to help eliminate the parasite by blocking its transmission, is a priority. The barriers to the development of these vaccines have been the shortage of clearly-identified immunogenic antigens for all the stages of the parasite life cycle, the absence of clearly-defined protection markers, a limited number of safe and effective delivery systems (adjuvants inducing a potent and lasting humoral or cellular immune response) and, for vaccines designed to attain herd protection targeting the developmental stages of the parasite or mosquito antigens, the absence of a pre-established clinical and regulatory roadmap to pave the way for vaccine licensure by the regulatory authorities.

Conclusions

Historically, successful vaccines have been effective against pathogens treatable with antibodies and with a stable antigen repertoire. HIV, malaria, and tuberculosis have broad antigen variability and require T-cell immunity to obtain protection against these diseases. The development of vaccines against these pathogens requires new approaches such as structural vaccinology (a branch of structural biology that is emerging as a promising platform for the identification of effective protective antigens) and systems biology (computational and mathematical modeling of complex biological systems).

Moreover, we are entering an era where the extended use of a vaccine requires more than only safety and efficacy data. Recommendations for the use of new vaccines will be considered in terms of implementation studies that determine the most effective forms of widespread use. Otherwise, the vaccines most likely to fail are the ones developed mainly for the poorest peoples of the world.

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