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Conference report

Exploring immunological mechanisms of the whole sporozoite vaccination against *P. falciparum* malaria

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ABSTRACT

Great progress has been made in the development of whole sporozoite vaccines including the manufacturing of cryopreserved *Plasmodium falciparum* sporozoites (PfSPZ) suitable for clinical application. Such whole sporozoites are being used for clinical studies of controlled human malaria infection (CHMI) as well as for evaluation of candidate vaccine approaches (both attenuated sporozoites and infectious sporozoites administered with chemoprophylaxis) and as reagents for immunology and cell biology assays. CHMI studies with whole sporozoites provide a great opportunity to better understand the intrinsic mechanisms of resistance to *P. falciparum* (*e.g.* due to sickle cell trait and other hemoglobinopathies) as well as host responses to an initial *P. falciparum* infection. High-level protective efficacy has been demonstrated in a small number of volunteers after intravenous (IV) inoculation of radiation-attenuated PfSPZ or in those who were exposed to live PfSPZ while on malaria chemoprophylaxis. These advances and data warrant further investigations of the immunological mechanism(s) whereby whole sporozoite inoculation elicits protective immunity in order to facilitate whole sporozoite vaccine development. The National Institute of Allergy and Infectious Diseases (NIAID) convened a workshop on Sept. 2–3, 2014 involving participation of international experts in the field of malaria vaccine development, and in basic and clinical immunology research. The workshop discussed the current understanding of host immune responses to whole malaria sporozoite inoculation, identified gaps in knowledge, resources to facilitate progress, and applicable new technologies and approaches to accelerate immunologic and vaccinologic studies and biomarker identification. This report summarizes the discussions and major conclusions from the workshop participants.

1. Introduction

Annually, there are over 198 million cases of malaria and approximately 584,000 deaths globally, with an estimated 3.2 billion people still at risk of being infected with malaria and developing disease [1]. A first generation malaria vaccine RTS,S/AS01B, a subunit vaccine incorporated into a virus-like particle, has demonstrated efficacy of 40–50% against clinical malaria in infants and children in a large, multicenter Phase III clinical trial. Although the RTS,S/AS01B vaccine efficacy is lower in infants than in children and wanes over time, the vaccine nevertheless appears headed towards licensure [2]. While efforts are on-going to better understand RTS,S/AS01B's properties and its underlying immunologic mechanism(s) of protection, the development of the next generation of malaria vaccines with much better efficacy is under active investigation. Currently, a radiation-attenuated *Plasmodium falciparum* sporozoite vaccine has demonstrated close to 100% sterile protection in a Phase I/IIa trial when administered intravenously (IV) to a small number of volunteers in a controlled human malaria infection (CHMI) study [3]. Multiple follow-on trials designed to replicate these findings with larger sample sizes are underway, with the ultimate goal of further defining the key vaccinologic and immunologic aspects. In addition, various human challenge models either by mosquito bite or by sporozoite inoculation *via* different routes [*e.g.* IV, intramuscular (IM), subcutaneous (SC), or intradermal (ID)] are being proposed as valuable tools for clinical immunology research or for vaccine evaluation. This convergent flow of new data, the advances in next generation vaccine development, and the increasing availability of novel technologies have led to several important questions for the malaria vaccine research community:

- What are the initial host immune responses at the skin upon sporozoite inoculation and subsequently in the liver after sporozoite infection of liver cells?
- What are the most important targets and mechanisms of protective immunity against pre-erythrocytic stages of *Plasmodium falciparum*?
- Does immune interference occur in field situations?
- How do we leverage animal models, CHMI, any other newly available tools or technologies, or field studies to accelerate basic understanding as well as towards the developmental effort for a highly efficacious whole sporozoite vaccine?
- Can we develop an efficient, coordinated, collaborative effort across multiple organizations to systematically design studies, analyze and objectively evaluate immune responses, and support an evidence-based approach to vaccine development?

The workshop was considered as timely and reflected the evolution of the science and of the convergence of two NIAID

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priority areas, malaria and vaccines. It was organized into an initial overview followed by three sessions, each addressing a high priority topic. The first session addressed adaptive immunity in the human response to malaria parasite inoculation; investigators presented data and described progress made in clinical immunology studies either in conjunction with different whole parasite vaccine evaluation or in human challenge settings. The second session dissected and explored the deeper understanding of whole parasite inoculation in animals and how the recent advances and knowledge obtained in these models could help to guide clinical vaccinology and immunology studies. The third session focused on the landscape of new technologies and tools and other new research findings that might benefit the malaria vaccine community. A summary of the workshop discussions follows.

2. Adaptive immunity in human to malaria parasite inoculation

2.1. Host responses after CHMI

The CHMI model involves infecting volunteers with parasite sporozoites in a controlled environment and then treating the volunteers with antimalarial drug(s) at a specific time-point to prevent further infection and disease development. A controlled exposure with five bites from laboratory-reared mosquitos carrying *P. falciparum* sporozoites could consistently achieve 100% infection of volunteers [4,5]. Dr. Robert Sauerwein reported that even a single mosquito bite inoculation in naïve adults in a CHMI setting could induce long-lasting adaptive immunity as demonstrated by long-term induction of IFN-γ producing cells and anti-circumsporozoite protein (CSP) antibody (Ab) titers [6,7]. In addition, a single CHMI inoculation in Tanzanian healthy adults [8] could induce significant boosting of functional-antibody-dependent merozoite opsonization activity (presented by Dr. Claudia Daubenberger). Molecular analysis of PBMCs of these volunteers showed that more than 2000 genes were differentially expressed across different time points, and that early gene expression profiles also appeared correlated with later B cell responses (presented by Dr. Raphael Gottardo). These limited studies demonstrated that CHMI, either by mosquito bites or needle inoculation, is a powerful technique to provoke potent immune responses against many antigens, both in immunologically naïve individuals and in those having had extended natural exposure to malaria in the field. Hence, the CHMI provides not only a simple model for better understanding of the disease process or for biomarker identification, but is also a powerful tool for gathering valuable immune baseline data that would be advantageous to future natural immunity studies and for whole sporozoite vaccine clinical evaluation.

2.2. Immunity after chloroquine prophylaxis and sporozoite (CPS) immunization followed by CHMI

CPS involves performing immunization with live sporozoites together with drug coverage to "attenuate" the parasites *in vivo*. Dr. Sauerwein summarized data indicating that CPS *via* mosquito bites under chloroquine coverage in a malaria naïve population could confer up to 100% homologous protection against CHMI challenge, that the level of protection was parasite inoculation dose-dependent [9], and that the protection was sustainable [10].

It was also pointed out that CPS-induced immune signatures in a differentially protected cohort indicated that Abs and memory B cells with specificity against both pre-erythrocytic and blood stage antigens were detected [11]. The protected individuals reacted more to pre-erythrocytic and "mixed" stage (both pre-erythrocytic and sexual blood stage) antigens, indicating that protection was mediated by pre-erythrocytic immunity. This was further confirmed by another IV challenge study with *P. falciparum*-infected erythrocytes in which none of the CPS immunized subjects was protected against the blood stage diseases [12]. In contrast, unprotected individuals showed more reactivity against blood stage antigens. In addition, a wide range of cellular responses were also detected, including IFN-γ secreting CD4, CD8 cells and semi-innate cells like γδ T cells [6]. In those completely protected individuals after CPS immunization, significantly higher percentage of CD107a $CD4^+$ T cells or granzyme B $CD8^+$ T cells were detected, suggesting the possible roles of CD4 and CD8 T cell responses in the protective pre-erythrocytic immunity [13]. Similar to CHMI that induces long lasting immunity, CPS immunization also induced long-lived Ab responses that recognized many antigens expressed in a *P. falciparum* protein array [11], and most of the responses were not boosted by further parasite challenge; the exception to this was those unprotected individuals who would respond strongly after challenge against many antigens that they did not initially reacted to. So far, there does not seem to be a definitive correlate of protective Ab or cellular immunity. It is possible that CPS-mediated protection is conferred by a profile of protective immune responses consisting of different arms of immune defense mechanisms and many different antigen specificities. Further studies focusing on the identification of combinatorial humoral and cellular signatures were proposed to address this issue.

2.3. Immunity after attenuated sporozoite vaccination followed by CHMI

Currently, the most advanced whole parasite vaccine candidate in development is the radiation-attenuated sporozoite (RAS) vaccine for *P. falciparum* (PfSPZ). In humans, RAS PfSPZ behaves similarly to live sporozoites for the first few days, *i.e.*, the RAS travel to and infect the liver. However, unlike live sporozoites which replicate inside the liver and release merozoites into the blood circulation leading to a new wave of blood stage parasitemia on days 6–7, the RAS PfSPZ vaccine parasites only remain metabolically active and produces protein for the first three days after infection and thereafter, the vaccine sporozoites terminate their replication cycle. Dr. Stephen Hoffman presented data from the first clinical trial of the vaccine *via* ID or SC inoculation in malaria naïve adults; the data showed suboptimal immunogenic and protective features with a very low but detectable level of Ab and IFN-γ producing cells [14]. A subsequent trial with a total of five IV inoculations of the RAS PfSPZ showed a dose-dependent protection efficacy, with six out of six volunteers at the highest administered dose being protected against homologous CHMI challenge, with two out of these six protected individuals still being protected after a second CMHI challenge five months later [15]. These protected individuals seemed to show relatively high levels of CSP- or PfSPZ-specific Ab titers and Inhibition of Sporozoite Invasion activities (however, no responses against other pre-erythrocytic or blood stage antigens *i.e.*, PfCelTOS, PfAMA1, PfSSP2/TRAP, PfLSA-1, PfEXP-1, PfMSP-1, and PfEBA175 were found). PfSPZ-specific multifunctional recall CD4 and CD8 T cells were also detected in the PBMCs. Further analysis indicated that protection appeared to be associated with an anti-CSP ELISA OD titer greater than 2000 after the third immunization (Steve Hoffman, data unpublished) or with higher levels of anti-CSP and MSP-5 Ab titers [15]. Hence, results from these two trials seem to indicate that direct IV inoculation may be a more effective way to deliver vaccine to elicit protective immunity than skin inoculation. Dr. Patrick Duffy described another on-going trial in Mali to evaluate RAS PfSPZ vaccine safety and efficacy in a natural infection setting. This trial will assess whether pre-existing immunity in an endemic area would alter the vaccine efficacy in the context of boosting or immune regulation, as well as conduct

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exploratory analyses to identify protective antigens through T cell or Ab screening and parasite sequencing. A number of questions related to protective antigen discovery were raised: for example, are there multiple targets of antigen-specific protection? Is priming for a dominant epitope-specific response different from that for subdominant epitope-specific response? Are there multiple arms of immune protection? Dr. Hoffman also presented a full detailed clinical development plan for the PfSPZ vaccine that includes four different stages of development, with the third stage consisting of a pivotal Phase III trial and the final stage of a large scale proof of principle effort for malaria elimination and vaccine licensure in the near future. He stated that to date two clinical trials (NCT01001650, NCT0144116) to test the RAS PfSPZ as a vaccine product have been completed and many are on-going. The future trials would need to involve study subjects of different immune status: non-immune, minimally-immune, and semi-immune, and may address protection against infection with different strains of parasites *via* CHMI, and/or in a natural infection setting. The workshop participants also emphasized the need to address the durability of protective immunity by conducting human challenge at the immune memory phase (rather than following current protocol which allows CHMI at 2, 3 or 4 weeks after the last immunization).

Another attenuated sporozoite vaccine strategy employs genetically attenuated parasites (GAP). Dr. Stefan Kappe presented the first Phase I trial of a *P. falciparum* GAP in which mosquito bite delivery was used [16]. Immunization with the double knock-out parasites Pf 2KO GAP (p52-/p36-) was shown to be safe and did not cause parasitemia at a low dose; however, at a higher inoculum dose, one volunteer developed blood stage infection. Interestingly, the parasite recovered from this infected individual still possessed the double gene deletion. Potent antigen-specific CD4 and CD8 T cells as well as antibodies that block 50–80% sporozoite infection *in vitro* were elicited [17]. These limited data thus support the idea of proceeding with a new and more complete GAP attenuation approach. Another early gene deletion was recently added to the double KO strategy, giving rise to a triple KO parasite Pf 3KO GAP (*p52-/p36-/SAP1-*). The Pf 3KO GAP can infect hepatocytes *in vitro* at levels comparable to a wild type parasites but fail to complete liver stage development in a humanized mouse model [18], and is anticipated to be clinically tested in the near future. Another double KO parasite Pf SPZ-GA1 with two newly identified genes b9 and slarp deleted was also mentioned during the workshop. The Pf SPZ-GA1 was shown to arrest the parasites from day 2 onwards in primary human hepatocytes and completely abort development after infection in mice engrafted with human hepatocytes [19]. Thus far, all of the available GAP vaccine candidates seem to arrest early in the liver during development. Despite numerous attempts, late-arrested GAP parasites still cannot be made successfully. Since RAS and GAP-attenuated vaccines utilize very different attenuation strategies that could involve very distinct metabolic pathways in the liver, it is possible that they would induce significantly different protective immune profiles. Direct comparison of these two vaccination strategies and the induced immune profiles were proposed to aid further understanding of protective antigens and protective immunity as well as to facilitate future strategies for sporozoite vaccine development.

In summary, immunological studies with live or attenuated sporozoite vaccines have just started, and data related to immunity as a result of whole sporozoite inoculation are still limited. CHMI studies with live sporozoites provide a great opportunity to better understand host immune responses to a first *P. falciparum* infection and innate resistance to *P. falciparum* (*e.g.* sickle cell trait and other hemoglobinopathies), and to accumulate baseline information for further understanding of immune interaction and regulation. Data from live CPS immunization are still not very clear. A combined humoral and cellular immune profile might reflect the correlates of protection for this particular vaccine. Further, for RAS PfSPZ immunization, Abs against CSP and MSP-5 correlated with protection. However, many immunological questions remain unanswered for RAS PfSPZ immunization such as, route of administration, immunization regimen, induction and maintenance of long-lasting protective immunity, protective antigen identification, heterologous protection, and the effects of pre-existing immunity and co-infection in the field. GAP immunization might be another very promising vaccine strategy since only a single inoculation by mosquito bite could lead to potent Ab and T cell responses. In addition, different protective mechanism may dominate in different individuals, since Ab was associated with protection in some individuals but not in others. It is also possible that multiple protective surrogates might be identified in the future, but they might not be the actual mechanism of protection. The workshop participants recognized the great opportunities to conduct many other whole sporozoite vaccine evaluations and clinical immunology studies, however, also agreed that many of the future trials would be planned and designed for safety evaluation, and thus might not be that useful for immune signature studies. The workshop participants also suggested that comparative studies with these three immunization strategies (RAS, GAP, and CPS), for example, in terms of antigen expression and presentation in animals or human gene expression profiling in small human clinical studies early on during development should be carried out, and strongly encouraged a unified and transparent development strategy, a culture of collaboration, and of sharing of resources and data.

3. Insights from studies of pre-erythrocytic antimalarial immunity in animals

The immunological studies for whole sporozoite immunization in experimental mice started in the early 1960s [20]. Although data obtained in mice and with mouse parasites cannot be fully replicated in the human setting and with human parasite, nevertheless, a mouse model is still a convenient tool to dissect the biological events following *Plasmodium* parasite inoculation.

Dr. Jerome Vanderberg described studies in mice that demonstrated that only a few of the many available sporozoites in the mosquito salivary gland are actually slowly released from the mosquito proboscis [21,22]. Anti-CSP Abs in the skin were shown to immobilize sporozoites or to form immune complexes blockage at the distal end of the mosquito proboscis, thus significantly reducing the number of sporozoites delivered into the host. The few released sporozoites could ultimately have diverse fates, such as: (1) reaching the blood and traveling towards the liver, and then being phagocytized by spleen macrophages or actively invading liver cells [21,23]; (2) staying in the skin to be degraded or differentiated into "liver stage" parasites (*i.e.*, express liver stage antigens) [22]; or (3) invading lymph vessels or traveling directly to lymph nodes [24]. Since a large number of attenuated sporozoites are needed for generating a high level of protection when delivered *via* the mosquito bites [25], direct injection of these attenuated sporozoites by needle in large number could be a more efficient immunization approach than by having a mosquito probing the skin and delivering the antigens; and IV delivery allowing the sporozoites to reach liver cells quickly (sinusoids, hepatocytes, Kuffer cells) [26] could be more effective than other parental routes in establishing parasite infection of the liver cells. However, potential lack of mosquito-derived "immune modulators" in the sporozoite vaccine delivered by needle injection might also open a future opportunity for the vaccine improvement with the use of adjuvants. Once sporozoites successfully infect liver cells, molecular events mediating host cell resistance to sporozoites as described by Dr. Maria Mota would follow. These events are liver sensor mechanisms driven by a type I IFN

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response against liver-stage *Plasmodium* infection, and activated by a newly identified pathogen-associated molecular pattern (PAMPs) *Plasmodium* RNA or other PAMPs including hepatitis C virus RNA [27]. Irradiated attenuated sporozoites might not elicit full sensor responses and any type I IFN inducer might work as an adjuvant for an RAS vaccine. While innate immunity in the liver warrants further exploration, the protective mechanism of T cell immunity in the liver is also far from clear. Dr. John Harty showed that protective CD8 T cells need to reach a certain threshold, for example, of over 1% of the Peripheral Blood Leukocytes for CSP-specific CD8 T cells in a subunit protein antigen immunization strategy, to provide sterile protection [28,29]. In addition, specificity seemed more critical than simply the quantity and not every potent T cell antigen would be protective [30–33]. Lessons learned from subunit vaccine studies may guide our thinking about potential protective liver T cell immunity elicited by whole parasite vaccines and about ways to improve whole parasite vaccine design and evaluation.

Animal studies also showed that the immunity elicited by whole sporozoite vaccine could be further improved by the use of adjuvant approaches. Dr. Moriya Tsuji showed that a glycolipid adjuvant, 7DW8-5, which can bridge the CD1d marker on DCs and TCR on iNKT cells and can induce early activation of circulating monocytoid DCs [34,35], could increase CSP-specific CD8 T cells and sterile protection efficacy when co-administered IM with irradiated sporozoites as compared to that found without the use of adjuvant or could result in a similar level of protection to that achieved by IV sporozoite inoculation. Another adjuvant-type approach, laser treatment, which has been extensively used in the cosmetic field to enhance blood permeability without tissue damage [36], significantly increased sporozoite-specific antibody and T cells responses in the liver and spleen (presented by Dr. Meixiong Wu). Finally, another interesting sporozoite vaccination strategy using the mouse parasite *Plasmodium berghei* as a naturally attenuated sporozoite vaccine platform for immunization against human malaria was proposed by Dr. Miguel Prudencio. *P. berghei*, known as not causing malaria in humans, was found to infect human hepatic cells *in vitro*, *ex vivo*, and *in vivo* in humanized mice, and it was unable to replicate in human RBCs. A transgenic *P. berghei* that expressed *P. falciparum* CSP could elicit CSP-specific functional Abs and cellular immunity in small animals, and could infect Rhesus hepatocytes *in vivo* (data unpublished). Promising preclinical data have been generated to support this transgenic parasite sporozoite (*P. berghei* expressing *P. falciparum* CSP) being clinically evaluated in the very near future. Important information might also be gained from other animal models, such as, the newly available humanized mice that supports *P. falciparum* hepatocyte infection and develops liver and blood stage diseases [37] or from non-human primates (NHPs) studies that allow for assessment of parasite effects on or responses of multiple organs or tissues. Employing fluorescently-labeled parasites, the humanized mouse model has been used to follow parasite movement and development *in vivo*, and be used to evaluate sporozoite vaccine safety (presented by Dr. Kappe). NHP studies also showed the kinetics of CD8 and CD4 T cells responses in the liver or PBMCs following RAS vaccination (presented by Dr. Kavita Tewari). In summary, at least in animals, a sporozoite vaccine might be further improved or enhanced by use of adjuvant agents or a new technology platform, resulting in new vaccine features or possible vaccination improvements, such as, reduction of the required number of injections, dose sparing, or providing an alternative route of immunization other than IV.

In conclusion, studies with animal models have revealed that various host defense mechanisms exist in the skin to counteract the initial sporozoite deposition from the mosquito's proboscis. For vaccination or immunization, a syringe may be more efficient for sporozoite delivery. When using CHMI in challenge studies to evaluate vaccine efficacy, IV injection of sporozoites as challenge materials may bypass the interaction with the host immune defenses in the skin (mainly mediated by Abs) as compared to that which occurs with natural mosquito bites; this could thereby lead to an under-estimation of vaccine efficacy. In the liver, the type I IFN signaling pathway was responsible for the resistance to parasite infection of the liver cells. The workshop participants also suggested that the role of T cells and the IFN-γ-mediated protective pathways as defined in animals needs to be clearly and thoroughly verified in the human setting, and further exploration of protective T cell antigens is needed. Finally, the immunity induced by sporozoites could be further optimized by adjuvants or adjuvant-like approaches. Transgenic *P. berghei* sporozoites expressing *P. falciparum* antigens offers a promising alternative platform to improve whole sporozoite vaccines. Knowledge obtained from sporozoite biology and immunity studies in animals including humanized mice or NHPs offers the potential for great insight and a better understanding about whole parasite vaccine design and evaluation.

4. New tools and advances in identification of biomarkers and correlates of protection

New technologies and tools have enabled immune profiling, biomarker and correlates of protection studies to be conducted in a high throughput (HTP) and systematic manner. One such technology is the *P. falciparum* protein microarray, which consists of an 8000 antigen repertoire instead of one or a few of antigens. Dr. Felgner pointed out that while protein array analysis had assisted in finding the protective association elicited by IV immunization with RAS PfSPZ [15], some trends of correlation with other antigens also exist, but a sufficient sample size would be needed to resolve these trends and for future studies.

Dr. Emily Smith presented a systematic approach to pre-erythrocytic antigen identification using IFN-γ producing T cells and Abs from protected *vs.* unprotected individuals and Gateway expression cloning/wheat-germ cell-free expression of *P. falciparum* proteins. The identified proteins were further confirmed in a *Plasmodium yoelli* mouse challenge model using mouse parasite homologous proteins [38], and also described another approach focusing on liver-stage antigen discovery involving eluting, separating, and characterizing host HLA molecule-presented parasite peptides from infected primary human hepatocyte cultures followed by further peptide presentation verification with PBMCs from RAS-immunized individuals. Another new technology, the ATLAS™ engine, to aid in new T cell antigen discovery in a HTP fashion was presented by Dr. Jean-Luc Bodmer [39]. The technology has several desirable properties for T cell antigen screening as it allows for full-length protein screening of the entire proteome with human autologous immune cells and measures immune parameters relevant to the diseases in an operationally manageable manner. A human *MIMIC* technology, which translates the human immune system into a biomimetic 3D *in vitro* system (http://www.vaxdesign.com/) and presents a great potential to conduct "an clinical trial in a test tube" was presented by Dr. Ernesto Luna. The *MIMIC* offers the advantage of reducing the number of subjects needed for clinical trials and removing donor-to-donor variability, and allows for the assessment of multiple vaccine formulations, determination of dose, and for the easy comparison of attenuation. The workshop participants recognized that many new antigens have been identified over the years yet information about these new antigens is not readily or publically available to the research community, and strongly emphasized the need for information exchange, antigen identity cross referencing, and confirmation and validation of approaches and new discoveries among different investigative groups and organizations. The participants also pointed out the

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importance of leveraging new protective antigen information for better assay design and development applicable to future protective immunity studies.

Systems biology, or systems immunology was also introduced by Dr. Damien Chaussabel as having a great potential for malaria immunology studies. It could help to determine if there is a specific pathway/mechanism or multiple factors or profiles responsible for any correlates or associations. With increasing development of new technologies and with large amounts of data being generated, the challenges reside in data integration and analysis. Several improvements to make systems immunology more approachable were pointed out, including developing easier methods for sample collection or for more frequent sample collection. Among approaches that would be valuable are: creating a new data analysis platform, such as working with a modular repertoire rather than studying individual genes; or undertaking dataset collection from a broad range of diseases and conditions for further clustering behavioral analysis and relationship determination [40–42]. In addition, the power of data integration and sharing in the era of systems immunology is enormous. Combining complementary data and projects with limited sample size would generate new opportunities to extract novel meaningful information. However, standardization and data open-access would be extremely critical for information sharing. While forming a consortium to capture all of the immunological activities may be worthwhile, the community should also consider the need for assay standardization and uniformity of data input and collection. Dr. Gottardo reminded the group about the NIAID-supported Human Immunology Project Consortium (HIPC), which has established a central database and analysis engine to capture datasets on many infectious diseases including malaria, and the web-interface, Immunespace (www.immunespace.org) might be a valuable resource for the malaria vaccine community.

In summary, new tools and technologies, such as protein arrays, new T cell antigen discovery technology, and a biomimetic 3D *in vitro* human immune system have either already provided or will present great opportunities to conduct systematic immunological studies in a HTP manner. A great deal of information and data will be collected; data integration and sharing among the members of the malaria research community would become extremely important and was highlighted as one of the key action items.

5. Summary

Development of malaria vaccines with protective efficacy of 75% against clinical malaria suitable for administration to appropriate at-risk groups in malaria endemic areas is one of the strategic goals in the WHO's malaria vaccine technology roadmap. The whole sporozoite based vaccine strategy, the RAS, the GAP, or the CPS may offer hopes in achieving such a highly efficacious vaccine. However, significant vaccinology and immunology issues still remain unsolved. Common challenges for all these strategies include eliciting protection against heterologous parasites, potential improvement of immunization regimens (number of doses and dosage, interval between dosing) and route of vaccination, immune mechanism(s) of protection or signature(s) of protection/non-protection, definition and measurement of memory and of mechanism(s) of durability, basic biology of multiple antigens and innate immunity in the skin and liver and how they shape adaptive immune responses, antigen processing and presentation and effector mechanisms, and new protective antigen identification. The workshop participants also repeatedly emphasized the importance of establishing a cooperative mechanism to compare the three different immunization strategies (RAS, GAP, and CSP) across different groups or organizations early on during vaccine development as a very important exercise to better understand whole sporozoite immunity leading to efficient sporozoite vaccine development. The CHMI was highlighted as a valuable tool for vaccine evaluation or basic immunology studies yet there is still insufficient information about the difference, if any, between challenge-by-mosquito bites and "challenge-in-a-bottle" with needle *via* different parental routes, and whether the method of challenge affects the assessment of vaccine efficacy.

Continued exploration of the immunological mechanisms of whole sporozoite vaccination could be benefit tremendously from newly available tools and technologies. Besides those technologies discussed at the workshop, such as protein array, T cell antigen discovery engine, and the human 3D mimics system, other cutting edge immunological tools like CyTOF technology and mass spectrometry for epitope identification were also identified as being potentially very helpful. Studies in mice or NHP are considered as valuable approaches to study basic biology, pathogenesis, innate immunity, and basic adaptive immunity so as to guide clinical understanding of whole sporozoite vaccination. Common themes to facilitate collaboration, resource sharing, and data comparison and to enhance data quality were also discussed, some examples of which included a library of well-designed and well-constructed *Plasmodium* antigenic peptides, readily available well-characterized sporozoites for use as laboratory reagents, an increased information transparency of newly identified protein antigens, and a readily available information resource for parasite genetic sequences. In addition, systematic approaches and processes to assess or down select adjuvants or adjuvant-like components for improving whole sporozoite vaccines or for new protective antigens suitable and relevant to vaccine development and evaluation are extremely important. Finally, more comprehensive tools/assays or various "omics" technologies to obtain and verify "big data" such as immune signatures would be critical in the future. Continued improvement in processing steps such as sample collection or analysis tool enhancement should also be encouraged as these will affect the quality of data and the informational output.

Given the substantial resource requirement for vaccine development to combat this globally important disease, collaboration and communication among groups/organizations at every level (investigators, funders, or other stakeholders) are critical. Bi-directional interactions among basic scientists and clinical investigators are important catalysts for moving this interdisciplinary field forward. Enhanced communication about trial design and conduct, immunization protocols, assays and techniques, and real-time sharing of new data are needed. There appears to be great enthusiasm from the community in sharing of translational research data, in particular for whole sporozoite vaccine trials and the related immunology studies. However, the community was also cautious about the difficulties inherent in sharing data in that standardized approaches (including trial design, assay formats, reagent panels, and nomenclatures, *etc.*) and even the availability of dedicated biomathematicians are critical in order to allow for comparability and accessibility.

In conclusion, the whole sporozoite immunization strategies mark the beginning of a promising future for a highly efficacious malaria vaccine. Further studies to better understand the immunological aspects would help to accelerate development of the whole sporozoite-based vaccines.

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Conflict of interest statement

The authors have no conflicts of interest to declare.

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Annie X.Y. Mo*
John Pesce
B. Fenton Hall
National Institute of Allergy and Infectious Diseases (NIAID), National Institute of Health, Department of Health and Human Service, 5601 Fishers Lane, Rockville, MD 20852, USA

* Corresponding author. Tel.: +1 240 627 3320; fax: +1 240 627 3467.
E-mail addresses: moa@niaid.nih.gov, anniexymo@gmail.com (A.X.Y. Mo).

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