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What can we learn from an unnatural immune response?

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Summary

In a recent review on CD8+ T cell responses after malaria infection and immunization, Zavala and colleagues clearly outline two decades of research and formulate the important questions that remain going forward. Here we discuss some of these findings and highlight their importance to malaria vaccinology.

Overview of the pre-erythrocytic stage vaccine effort

The life cycle of *Plasmodium* is complex, involving both mosquito and mammalian hosts and several distinct stages, each with its own set of antigens. In the mammalian host, the infective sporozoite and the resulting liver stages or exoerythrocytic forms (EEFs) establish infection and constitute the asymptomatic pre-erythrocytic stage. This is a time when parasite numbers in the host are low and their eradication can abrogate infection. The majority of people living in endemic areas do not generate a completely effective immune response to pre-erythrocytic stages and are susceptible to malaria infection throughout their lives [1,2]. Since one successful sporozoite can give rise to a full-blown malaria infection, a vaccine targeting pre-erythrocytic stages is expected to induce sterilizing immunity.

Forty years ago it was shown that high doses of irradiated sporozoites induce sterile immunity in mouse models of malaria and in humans, demonstrating that sterilizing protection to pre-erythrocytic stages is feasible (reviewed in Ref. [3]). Since sporozoites must be dissected from mosquito salivary glands and cryopreserved, a whole parasite vaccine seemed impractical and a subunit strategy was pursued. The major surface protein of the sporozoite, circumsporozoite protein (CSP), is the dominant antigen in the protective response to irradiated sporozoites [4] and has been the major focus of the preerythrocytic stage vaccine effort. This work has culminated in the ongoing Phase III trials of RTS,S, composed of the CSP repeat region and carboxy-terminus fused to hepatitis B surface antigen [5]. To date, the efficacy of RTS,S is between 29 and 65 percent when protection from re-infection is the measured outcome [5], with the range likely due to the different adjuvants used or age groups tested in each trial. Recently, impressive progress has been made in overcoming the technical hurdles involved in generating FDA-grade sporozoites and whole parasite vaccines are now in clinical trials [6]. This effort has been boosted by the generation of genetically-attenuated sporozoites that confer protection similar to that observed with irradiated sporozoites [7].

Nonetheless, given the resources required to reach individuals in resource-poor settings with a whole parasite vaccine and the limited efficacy and duration of RTS,S, it remains important

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to better understand the nature of the protective immune response generated by irradiated sporozoites and attempt to translate these findings into an efficacious vaccine.

Protection conferred by irradiated sporozoites is due to antibodies targeting sporozoites and cytotoxic T cells recognizing infected hepatocytes (reviewed in Ref. [3]). Although antibodies to CSP can inhibit sporozoite infectivity and are likely an important component of the protection afforded by RTS,S, complete inhibition requires high titers of high affinity antibodies that are difficult to obtain. Therefore, T cell responses to infected hepatocytes are thought to be a critical component of an effective immune response to pre-erythrocytic stages. Zavala and colleagues have reviewed the salient features of this response [8], and here we highlight their major points and discuss their relevance to the malaria vaccine effort.

Generating protective T cell responses to pre-erythrocytic stages

The goal of any vaccine is to generate long-lasting immunological memory to a pathogen such that upon re-exposure, the response is rapid and complete. However, in contrast to immunization with irradiated sporozoites, individuals living in malaria-endemic areas generally do not have robust, enduring T cell responses to pre-erythrocytic stages [9]. Recent data from mouse models have found that protection requires malaria-specific CD8⁺ T-cells to exceed 1% of circulating lymphocytes, which is greater than what is required for other pathogens and what is observed after vaccination with RTS,S [10]. This may be explained by the biology of pre-erythrocytic stages which requires that CD8⁺ T cells patrol the entire liver to kill a small number of infected hepatocytes in a short time. How do we achieve this level with vaccination? Although there does not appear to be a universal mechanism to generate effective memory, some critical factors include: (i) the initial dose of antigen, (ii) the duration of encounter with antigen and (iii) the context of presentation by antigen-presenting cells (APCs) [11].

Sporozoite dose

Using a rodent model of malaria, it was found that a single heavily infected mosquito injects, on average, 125 sporozoites [12]. In the field, where mosquitoes have lighter infections, the inoculum is likely five-fold lower [13]. People living in hyperendemic regions can receive several infective mosquito bites per week resulting in a yearly dose of 1500 to 15,000 sporozoites, a level of exposure that generally does not result in a protective immune response. In contrast, immunization by the bites of 1000 heavily infected mosquitoes (~125,000 sporozoites) over several weeks does lead to sterile protection [14]. Over a lifetime, a person living in a malaria-endemic region can indeed be exposed to such large numbers of sporozoites; however, the prolonged timeframe of exposure impacts the nature of the immune response. Indeed, in other systems, low levels of antigen over a long period of time can lead to tolerance rather than accumulated memory responses [15]. Rodent models indicate that the initial sporozoite dose determines a baseline CD8⁺ T-cell memory response and subsequent doses do not increase this response [16]. Therefore, antigen dose and the time over which it is administered are key to generating a large pool of effector memory cells that can confer protection to subsequent challenge.

Location

For years it was assumed that sporozoites rapidly targeted the liver after inoculation and that antigen presentation and T-cell priming occurred in this location despite this organ's tolerogenic properties. We now know that mosquitoes inoculate sporozoites into the dermis where they spend several hours migrating and traversing cellular barriers before encountering and invading a vessel [17,18]. Additionally, in rodent models 20% of the inoculum goes to the draining lymph node (dLN) where some sporozoites can remain and begin to develop for up

to 24 hours before being destroyed [17,18]. Elegant work using intradermal injection or the bites of infected mosquitoes clearly shows that the immune response in mice is initiated in the dLN, where CD8+ T cells are primed and subsequently migrate to the liver to kill infected hepatocytes [19]. Importantly, successful immunization of humans with irradiated sporozoites has always been performed by mosquito bite, delivering sporozoites intradermally. It is possible, therefore, that the parasite's presence in the skin and dLN are important in the generation of a protective immune response and this should be considered in choosing a vaccine delivery route.

The context of antigen presentation

The initial antigen encounter and the innate molecules involved are critical in shaping the adaptive immune response. Important questions at the intersection of sporozoite biology and immunology arise from this, namely: (i) how and where do professional APCs acquire sporozoite antigens, and (ii) what is the identity of these APCs? Studies have shown that heat killed sporozoites are ineffective at inducing an immune response whereas live sporozoites induce a robust response [20]. Live sporozoites are actively motile, a property required for exiting the skin, and they traverse different cells in the dermis, including potential APCs [21]. Thus APCs in the skin may acquire antigen and migrate to the dLN for T cell priming. Alternatively APCs in the dLN may acquire antigen from the sporozoites that go to this location. Previous data demonstrates that CD11c+ dendritic cells (DCs) are critical for the initiation of T cell responses to CSP after immunization by irradiated sporozoites [19]. In addition to lymph node-resident DCs, there are CD11c+ cells in the skin, namely resident Langerhans cells and dermal DCs. Determining which population of DCs is critical for antigen presentation and dissecting how antigen is acquired, are now feasible with new tools such as non-motile sporozoite mutants and mice deficient in molecules involved in dendritic cell trafficking and development.

Selection of antigens: is CSP a red herring?

Although CSP is the immunodominant antigen in the T cell responses to irradiated sporozoites [4], there are good reasons to think beyond CSP. First, the modest efficacy of RTS,S stands in contrast to the protection induced by irradiated sporozoites and suggests that CSP alone may be insufficient to induce sterile protection. Second, in contrast to other pathogens that can initiate infection with a low inoculum but replicate exponentially over time, the sporozoite is a nonreplicative form and so an immune response to it is quickly rendered ineffectual as sporozoites invade hepatocytes and transform into EEFs. During EEF development CSP expression ceases, and a new array of proteins is expressed [22]. Thus a CSP-specific response may not recognize and kill infected hepatocytes that have escaped early destruction. Given that late stage EEF antigens are expressed in a relatively immunotolerant organ, one wonders whether the immunodominance of CSP has evolved to divert the hosts' response from late stage antigens. Therefore it seems prudent to identify other liver stage antigens that could be combined with CSP to generate an efficacious vaccine. This effort has been facilitated by the recent proteomic and transcriptional profiling of rodent parasite liver stages [22], and identifying additional proteins capable of generating effector T cells may now be possible. To this end, another model of pre-erythrocytic stage immunity may be useful. It has been shown that live sporozoites administered under chloroquine cover also led to protective immunity in mice and humans [23,24]. Unlike irradiated sporozoites which are arrested in early development, chloroquine does not kill liver stages or early blood stages, allowing for full development of EEFs and initial seeding of the blood. Although a portion of the immunity generated by this protocol targets blood stage parasites [24], it is likely that EEFs are also targeted. Thus, this system may enable the identification of late-stage EEF antigens that contribute to a protective immune response.

How to boost cellular immunity?

Heterologous prime-boosting strategies can generate potent cellular immunity. Although T cells will develop both to the priming and boosting vectors, memory T cells to the shared target antigen are selectively expanded. In rodent models of malaria, priming with irradiated sporozoites and boosting with recombinant vaccinia virus encoding a CSP epitope induces better expansion than a homologous boost with sporozoites [16]. Using TRAP, another sporozoite antigen, Hill and colleagues are investigating different primeboost strategies in malaria-naïve individuals and finding that priming with fowlpox virus and boosting with vaccinia leads to CD8+ T cell expansion [25]. These data have important implications for vaccinating non-naïve populations where low inocula may set a low T cell memory baseline or possibly induce a tolerogenic response to sporozoite antigens. Indeed, people in malaria-endemic areas have low levels of cytotoxic T cells to sporozoite antigens that would have to be expanded [9]. Although prime-boost strategies do not boost CD8+ T cells to the protective threshold described by Schmidt et al. [10], ultimately it is the T cells that go to the liver and destroy infected hepatocytes that are important, and this number may vary depending upon the immunization protocol. Another possible way to augment the cellular immune response is to target infants that have not yet been significantly exposed to sporozoites. Indeed, recent data from Phase IIb trials with RTS,S in infants demonstrated significantly higher efficacies compared to what was observed in adults and children [5]. Phase III clinical trials of RTS,S will be performed in infants aged 6 to 12 weeks or 5 to 17 months, and it will be informative to study the types and duration of immune responses in these distinct groups.

Conclusion

The review of T cell responses to pre-erythrocytic stages of *Plasmodium* parasites by Zavala and colleagues raises several critical issues important to the malaria vaccine effort [8]. In contrast to highly successful vaccines, such as those for smallpox and yellow fever, a purely empirical approach is unlikely to work for malaria and the insights gained from the studies reviewed by Zavala and colleagues, should enable malaria vaccinology to move forward in a rational manner.

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