Vaccine xxx (2016) xxx–xxx

Contents lists available at ScienceDirect

Vaccine

journal homepage: www.elsevier.com/locate/vaccine

Review

Route of administration of attenuated sporozoites is instrumental in rendering immunity against Plasmodia infection

Rajesh Parmar[1], Hardik Patel[1], Naveen Yadav, Manoj Patidar, Rajeev K. Tyagi, Sarat Kumar Dalai*

Institute of Science, Nirma University, Ahmedabad, Gujarat, India

ARTICLE INFO

Article history:
Received 2 November 2015
Received in revised form 19 April 2016
Accepted 28 April 2016
Available online xxx

Keywords:
Radiation attenuated sporozoites (RAS)
Circumsporozoite (CS) antigen
$CD8^+$ T cells
Liver-stage (LS) antigen
Plasmodium berghei
Liver-stage malaria vaccine

ABSTRACT

Whole sporozoite vaccine (WSV) approach has been shown to induce efficient $CD8^+$ T cell response, critical for developing of long-lasting sterile protection against *Plasmodium*. Although WSV was initiated over four decades ago, we still do not fully understand about the absolute requirements for the generation of liver-stage specific $CD8^+$ T memory cells. For more than a decade intravenous (IV) route of immunization has been shown to be protective in pre-clinical studies. However, the intradermal (ID) route is preferred over IV route by many researchers as it is perceived to mimic the natural route of parasite delivery through mosquito bite. Various clinical studies have shown that ID route provokes poor protective responses compared to those seen with IV route of administration. The present study highlights the importance of circumsporozoite (CS) protein in preventing sporozoite entry to the hepatocytes, which however, it is not necessarily sufficient to ensure sterile protection. Instead, this article favors the idea that liver-stage development is a prime requirement for generation of antigen specific $CD8^+$ T cells and suggests the conditions favored by IV inoculation of sporozoite.

1. Introduction

Despite substantial efforts have been made to develop anti-malarial vaccine, however, malaria still remains the challenge to human health claiming millions of lives each year [1]. RTS, S/AS01 (MOSQUIRIX), the only pre-erythrocytic sub-unit vaccine against *Plasmodium falciparum*, comprising critical antigenic peptide from circumsporozoite (CS) protein has been shown to be promising, however, its efficacy and durability are lower than expected [2,3]. Considering the present scenario of RTS, S vaccine the integration of non-CS liver-stage (LS) antigen(s) would be an important requirement for developing next generation anti-malarial vaccine. The knowledge acquired through the preclinical studies and human trials of whole sporozoite vaccine (WSV) would help facilitate the process. As multiple immunizations with radiation-attenuated sporozoites (RAS) have reportedly [4,5] rendered sterile protection against LS *Plasmodium* infection, WSV approach remains the epicenter of anti-malarial therapeutic intervention program. One of the obvious limitations of WSV development program is the route of sporozoite administration. Since early studies [5–7] have shown that delivery of RAS through mosquito bite induces nearly or complete sterile protection, the general notion is that delivery of sporozoite through mosquito bite could be mimicked by intradermal (ID) route of inoculation in order to induce sterile protection. Surprisingly, even after four to six immunizations with RAS through ID route animals and/or human subjects scarcely achieved sterile protection when challenged through *P. falciparum* sporozoites infected mosquito bites (PfSPZ) [8]. On the contrary, studies dealing with intravenous (IV) route of administration with sporozoites in animals and humans have consistently shown to confer sterile protection [8,9]. This has provoked the debate whether or not IV route should be preferred over ID. We thought the sub-optimal protection following ID route of administration is achievable because of the generation of lower magnitude of $CD8^+$ T cell responses against LS infection of *Plasmodium* species. Moreover, the frequency of $CD8^+$ T cells is not considerably elevated to match the frequency generated through IV route of administration even after increasing the number and schedule of dosages.

In the present article we attempt to address some of aforesaid issues that pertain to evoking the robust immune responses required to protect the host by interrupting LS development of the parasite. We have included several viewpoints based on the findings from various studies including that of ours, and have come up

* Corresponding author at: Institute of Science, Nirma University, Sarkhej-Gandhinagar Highway, Ahmedabad 382481, Gujarat, India. Tel.: +91 79 30642753.
E-mail address: sarat.dalai@nirmauni.ac.in (S.K. Dalai).
[1] Co-first authors.

http://dx.doi.org/10.1016/j.vaccine.2016.04.095
0264-410X/

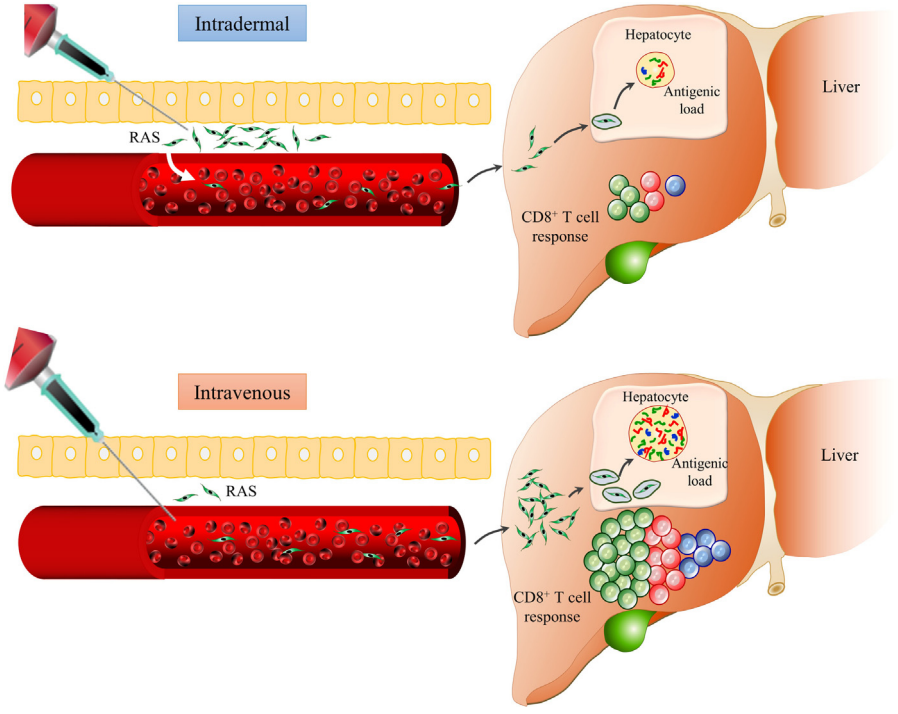

Fig. 1. Association between antigen load and frequency of liver stage antigen specific CD8⁺ T cells. RAS inoculated through ID route induces less number of antigen specific CD8⁺ T cells compared to that with IV route. Majority of sporozoites are seen trapped in the dermis and only fewer sporozoites invading the liver will result in lesser amount of liver-stage antigens. On the contrary IV route of administration of sporozoites are efficiently targeted to the liver, and antigenic load to activate CD8⁺ T cells would be higher.

with the following mechanistic explanations that might involve the induction of sterile protection.

2. Liver-stage antigen load and generation of CD8⁺ T cells

Earlier neutralizing antibodies have been described as the crucial player for generating protective immunity [10]. While the potent antibody response are generated by RAS immunization, other studies dealing with experiments like adoptive transfer or depletion of T cells suggest that LS antigen specific CD8⁺ T cells are instrumental in clearing infected hepatocytes to ensue sterile protection [11–14]. It has been suggested that CD8⁺ T cell frequency must reach to minimum threshold (1% of total PBMCs) to hinder progression and development of parasite from liver to blood stream [15]. Interestingly, the route of administration of RAS appears to modulate the frequency of antigen specific CD8⁺ T cells that correlates with the level of protection [16]. The intravenous route of inoculation of RAS has been shown to induce higher level of CD8⁺ T cells as compared to that seen with ID inoculation [8] in rodent and non-human primates, prior to mice were challenged with infectious sporozoite, IV immunized mice were completely protected whereas ID immunized mice were barely protected [16]. The inoculation of 'attenuated' sporozoites via different routes modulating the T cell responses is yet to be experimentally unraveled.

It is well established that density of MHC/peptide complexes available to the T cells decides the size of antigen specific T cells [17]. Although CD8⁺ T cell expansion is more efficient compared to that of CD4⁺ T cells because of less requirement of MHC/peptide complexes, the load of antigens available to the immune system would be an indispensable component to decide the outcome. In vivo real time imaging of parasite suggested that IV inoculation of 'infectious' sporozoites result in an accumulation of parasite with significantly higher load (approx. 30–50 fold) in liver compared to ID or SC administration [16,18]. These results are further supported by quantitative PCR analysis of parasite specific 18s rRNA which demonstrates that ID inoculation of *P. yoelii* sporozoites results in 10–20 fold lower parasite load in liver as compared to IV inoculation [19]. It is noteworthy that after mosquito bite, ~60% sporozoites trapped at the site of bite, ~15% enter lymphatic vessels, and nearly 25% of injected sporozoite cross the blood vessel and may finally reach liver [20]. Therefore, one might imagine that RAS injected through ID route would result into the reduced antigenic load in liver which is reflected by the poor T cell response (Fig. 1). The magnitude of T cell responses has been increased with increasing doses suggesting that higher antigenic load is required for inducing protective response [8], a condition fulfilled by IV inoculation of RAS. The latter is also supported by the findings in CPS (chemoprophylaxis and sporozoites) studies where sporozoites were inoculated through IV or ID route under chloroquine pressure [16].

The requirement of higher number of sporozoites for achieving ID induced protection confirmed the fact that parasite load in liver is inevitably required for the induction of sterile protection. Interestingly, many studies including ours demonstrated that mice primed with one higher dose of *P. berghei* sporozoites (IV route) and challenged with infectious sporozoites came down with the infection that is explained later. However, the mice which were immunized with three doses with relatively less numbers were completely protected from the infectious challenge [16]. In essence, multiple doses of immunization may not only take care of need of higher level of antigen but also allows the antigen to be presented in sustained manner for a given duration that would help developing sterile immunity.

3. Persistent antigen: a requirement for robust immune response

People in malaria endemic area develop protective immunity after several years of repeated exposure [21,22]. Interestingly, the acquisition rate of this protective immunity is found to be varying in different endemic settings. While those living in high transmission

Please cite this article in press as: Parmar R, et al. Route of administration of attenuated sporozoites is instrumental in rendering immunity against Plasmodia infection. Vaccine (2016), http://dx.doi.org/10.1016/j.vaccine.2016.04.095