

tides are linked to a lattice matrix of lysines [7-9]. Later constructs (TB4 and BT4) contained four copies of both the B cell epitope and a T cell epitope KQIRDSITEEWS (SEQ ID NO 4). While these constructs induced a protective (~80-100%) immune response to sporozoite challenge, the MAPS had to be emulsified with Complete Freund's Adjuvant (CFA) or Incomplete Freund's Adjuvant (IFA) before delivery. When mice were immunized with MAPS adsorbed to alum, the induced antibody titers were only about 25% of titers achieved with CFA delivery. Immunization with the MAPS combined in buffered saline, without adjuvant, elicited only minimal antibody titers and did not induce a protective immune response.

[0012] Particulate antigens are generally more immunogenic than non-particulate antigens. In recent years it has been recognized that particulate antigens such as virosomes [10-12], immunostimulating complexes (ISCOMS; [13, 14], PLG microparticles [15], and virus-like particles (VLP) [16-19] generally induce more effective humoral and cellular immune responses than those induced by soluble antigens. The VLP is a subunit vaccine that contains one or a few structural proteins of a virus that self-assemble into highly organized particulate structures. Incorporation of epitopes into the virus protein provides a way to deliver the epitope as an immunogen. The disadvantages to VLP are: 1) preexisting immunity to the virus may inhibit its use as a vaccine; 2) some VLP are large in size and their uptake by the immune system's dendritic cells may be difficult; and, 3) the use of the VLP may cause an undesired or preferential immune response to the VLP proteins which may in turn reduce the desired immune response to the vaccine epitope. Another major disadvantage of VLPs are that they are much less well understood with regard to their flexibility for tolerating modifications without disruption of the capsid-like structures.

[0013] Several VLP platforms have been tested in malaria vaccine development. Schodol made recombinant Hepatitis B core antigen (HBcAg) VLP incorporating the immunodominant B cell epitopes for *P. falciparum* (NANP)₃ (SEQ. ID NO.93), *P. berghei* (DPPPPNPN)₂, (SEQ ID NO 3) and *P. yoelii* (SYVPSAEQI) (SEQ ID NO 5) [20, 21]. The resulting hybrid HBcAg-CS proteins were particulate but required CFA, IFA, or alum for immunogenicity. Oliveira-Ferreira [22] put the CD8⁺ cell epitope of the *P. yoelii* CSP into the yeast VLP from retro-transposon Ty and attempted to immunize mice without adjuvant. The construct (TyCS VLP) was either preceded or followed by a dose of recombinant vaccinia virus expressing the entire *P. yoelii* CSP (VacPyCS). TyCS VLP or VacPyCS on their own induced undetectable or minimal T cell responses. Only the combination of TyCS VLP followed by VacPyCS was effective in induction of CSP specific CD8⁺ T cells capable of reducing the amount of plasmodial parasites, and at best only 62% of mice challenged were protected. Two immunizing doses of TyCS VLP in PBS had no detectable effect. Plebanski [23] cloned the *P. berghei* cytotoxic CD4⁺ T cell epitope (SYIPSAEKI) (SEQ ID NO 6) into the Ty vector. Constructs containing one or two epitopes administered intravenously at 100 µg/mouse in PBS induced good CTL responses but could not, on their own, induce a protective response to sporozoite challenge. Only upon heterologous boosting with a vaccinia construct containing the *P. berghei* CSP epitope was a protective immune response induced. The VLP construct did not have the capacity to boost the immune response by a second or third dose of VLP. Another PfCSP based vaccine, ICC-1134, containing both T-

and B-cell PfCSP epitopes in a modified Hepatitis B Virus core particle [24-26] was shown to be immunogenic if mixed with Montanide ISA-720™ (Ste D'exploitation De Produits Pour Les Industries Chimiques-S.E.P.P.I.C. Corporation Quai D'orsay 75321 Paris Cedex 07 France). However, multiple doses produced undesired adverse events in primates, and therefore only a single injection was used in a Phase I/IIa study resulting in minimal immunogenicity and no protection to sporozoite challenge. RTS,S, the *P. falciparum* CSP based vaccine, is a formulation of a VLP and the PfCSP protein fused with Hepatitis B Surface antigen, mixed with unfused Hepatitis B Surface Antigen in a proprietary combination and formulated with AS02A adjuvant. In multiple clinical trials and two field trials the vaccine has consistently only protected about 40% of vaccines from infection and the protection seems to wane after about 6 months. The vaccine is not a true VLP but more a mixture of about 75% Hep-Surface Protein and a PfCSP-HepB Surface Protein fusion that when mixed forms a particulate antigen.

Importance of Particle Size

[0014] Lymph node uptake: While it was previously thought that lymph nodes contained only mature DC incapable of further processing it has been recently proven that a substantial fraction of DC in the lymph nodes are immature and still capable of internalizing and processing antigen [27-29]. It has been determined that one of the important requirements for lymphatic system uptake from the interstitial space is particle size. Small particles (<40 nm) are quickly and easily taken up by lymphatic vessels [30]. ID injection of 20 nm particles are rapidly and highly efficiently taken up by lymphatic vessels, and retention in lymph nodes lasts for up to 120 h post-injection [31].

[0015] Epitope density and Ig cross-linking. It was noted early on in immunology that small organic molecules were not immunogenic, average sized proteins were only a little immunogenic, while protein complexes could elicit a stronger immune response. Larger, well ordered protein assemblies like VLPs [32] belong to the strongest immunogens that are known, especially if they repetitively display an antigenic epitope [33, 34]. The correlation of the size of the immunogen along with the density of the displayed antigen with the strength of the immune response is very difficult to establish. Nevertheless, decades ago Dintzis et al. demonstrated that such a correlation existed and that the spacing between epitopes was critically important for the strength of the immune response [35-37]. The organization of proteins on viral capsid structures increases the immune response significantly as opposed to the single soluble proteins [38]. More recently, Liu and Chen [39] have shown that antibody affinity constants are as much as 2 logs higher when antigens are displayed in optimal density arrays.

[0016] Thus there is a need for an inexpensive malaria vaccine that will prevent the death and debilitation of millions annually. Such a vaccine would also be useful widely for the existing populations as well as tourists, visitors, and also government and medical workers, refugees and other displaced people, soldiers and peacekeepers, and others who are deployed on humanitarian missions to malaria endemic areas, particularly Southeast Asia, Africa, as well as Central and South America.

BRIEF DESCRIPTION

[0017] The present invention is directed to self-assembling polypeptide nanoparticles, and to methods of using these nanoparticles for the diagnosis and treatment of malaria.