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Original article

Chimeric epitopes delivered by polymeric synthetic linear peptides induce protective immunity to malaria

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Abstract

Polymeric linear peptide chimeras (LPCs) that incorporate *Plasmodium vivax* promiscuous T cell epitopes and the *P. falciparum* circumsporozoite protein B cell epitope have been shown to induce a high level of immunogenicity and overcome genetic restriction when tested as vaccine immunogens in BALB/c mice. The present study evaluates the biological relevance of several LPCs using a well characterized rodent malaria model. Polymeric peptide constructs based on *P. berghei* and *P. yoelii* sequences, and orthologous to the human malaria sequences included in the original LPCs, were designed and tested for immunogenicity in mice of different H-2 haplotypes. We demonstrate that robust immune responses are induced and that peptides containing the orthologous rodent *Plasmodium* sequences exhibited similar immunogenic capabilities. Unique to this report, we show that LPCs can also prime MHC class I–restricted cytotoxic T lymphocytes (CTLs) and, most relevantly, that a peptide construct prototype incorporating single B, T and CTL epitopes induced protection against an experimental challenge with *P. berghei* or *P. yoelii* sporozoites. Collectively, these results suggest that polymeric polypeptide chimeras can be used as a platform to deliver subunit vaccines.

Keywords: Vaccine; Delivery; Malaria; Protection; Peptides

1. Introduction

Worldwide, malaria is the most significant vector-borne disease and, along with HIV/AIDS and tuberculosis, is at the top of the World Health Organization list of the most lethal transmissible entities [1]. The number of reported clinical cases of malaria ranges between 300 to 500 million, with one to five million fatalities each year [2]. This trend has remained stable in the last decade even after the introduction of innovative control measures [3]. In fact, the resurgence of the disease in geographical areas where it had been controlled has caused some authors to classify malaria as a re-emerging disease [3]. This global malaria crisis requires the active development and implementation of control programs including the increased use of insecticide treated nets, vector management, prompt and thorough treatment, and the introduction of effective vaccines.

The development of vaccines against the two most predominant human malaria species, *Plasmodium falciparum* and *P. vivax*, remains a major challenge. Malaria parasites contain over 5,000 genes and have a complex life cycle [4,5]. They also have much diversity and undergo antigenic variation [6]. Further these major species are evolutionarily distant, so a vaccine against one will not be effective against the other. Considering the diversity and complexity of *Plasmodium*, the intricate mechanisms involved in protection, many

Abbreviations: B, B cell epitope; CTL, cytotoxic T lymphocytes; CSP, circumsporozoite protein; IFA, indirect immunofluorescence assays; IFN-γ, interferon gamma; IL-4, interleukin 4; LPC, linear peptide chimera; MSP-1, merozoite surface protein 1; mAb, monoclonal antibody; O.D, optical densities; Pv, *Plasmodium vivax*; Py, *Plasmodium yoelii*; Pb, *Plasmodium berghei*; T, T cell epitope.

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of which remain unknown, malaria vaccine development faces various hurdles. Nevertheless, it is known that protection can be achieved through vaccination [7]. The ideal malaria vaccine will be cost effective to produce, stable and easy to deliver, and contain an adequate number of essential epitopes to induce long-lasting protection. We argue that efficient delivery systems for malaria vaccines must include parasite universal T cell epitopes, offer the flexibility to incorporate multiple epitopes in chimeric conformations, and be polymeric by nature.

Naturally acquired immunity to malaria is obtained after repeated infection and involves the targeting of an unknown number of antigens and the active involvement of T helper lymphocytes. Although thirty-five different clinical trials of malaria vaccines are currently in progress [8], the vaccine candidates tested thus far are mainly based on single stage-specific antigens. On the other hand, there is strong agreement that a multiepitope, multi-antigen vaccine will be important for malaria [5]. Towards this aim, subunit vaccines have been given much attention in the past decade, and the recent publication of a malaria vaccine Phase 2b clinical trial showing limited efficacy of a pre-erythrocytic chimeric antigen has focused attention on such possibilities [9]. Breakthroughs in delivery systems to greatly improve upon and optimize the make-up and functionality of malaria vaccines remain critical. As the search for effective malaria vaccines continues, the simplicity of modern procedures to synthesize peptide chimeric constructs makes them an attractive means to create multi-epitope immunogens.

We aim to design highly immunogenic and protective multi-epitope peptide chimeras based on simple synthetic procedures. It is well known that the immunogenicity of short synthetic peptides can be improved by the use of complex branched polymers with intricate stoichiometry, known as dendrimers. The synthesis of dendrimers requires the use of stepwise reactions that demand multiple quality controls. A different approach to improve the immunogenicity of synthetic peptides involves the use of exogenous carrier proteins that can provide extrinsic help. We have developed polymeric linear peptides chimeras (LPCs) as an affordable way to synthesize immunogenic peptides. LPCs have a very simple topology that includes the linear synthesis of a single malaria promiscuous T cell epitope in tandem with a B cell epitope, with a cysteine residue at both the amino and carboxy terminals (i.e. cys-T-B-cys). The rationale for using this strategy was to promote the formation of disulfide-linked versions of the chimeric peptides with a variety of molecular weights that would increase the density of individual epitopes. The addition of cysteine residues at the termini of synthetic peptides was originally described 15 years ago for the design of a complex *P. falciparum* chimeric vaccine candidate [10], while the LPC design is a unique recent advancement of this concept [11]. Six promiscuous T cell epitopes from the *P. vivax* Merozoite Surface Protein 1 (PvMSP-1) antigen have been described by us and used in the synthesis and evaluation of LPCs reported to date [11]. We have demonstrated that these LPCs are 200 to 4,000 fold more effective in the induction of antibodies in comparison with comparable peptides containing a genetically restricted T cell epitope [11]. Furthermore, cellular immune responses induced by immunization with these LPCs are long lasting; exhibit both T helper 1 and T helper 2 cytokine profiles, and are detected in the absence of ex vivo expansion. In this paper, we report the design and evaluation of a new set of LPCs based on orthologous rodent *Plasmodium* sequences, and demonstrate that these peptide constructs can simultaneously deliver T, B and CTL epitopes, and also induce protective immunity. Our data therefore strongly supports the continued investigation of LPCs as an alternative approach for delivering malaria vaccine components.

2. Materials and methods

2.1. Mice and immunizations

BALB/c (H-2^d) and C57BL6 (H-2^b) mice were obtained from Charles River Laboratories (Wilmington, MA). C3H (H-2^k), A/J (H-2^a) DBA (H-2^q), and SJL (H-2^s) were obtained from Jackson Laboratory (Bar Harbor, ME). All mice were females, 8-10 weeks of age at the time of the first immunization. The mice were housed in micro-isolation cages and all procedures were approved by Emory University's Institutional Animal Care and Use Committee and followed accordingly. Based on dose-response experiments we selected 50 μg as the optimal dose for immunization with peptide constructs. Groups of six mice were inoculated s.c. with individual peptides emulsified in Montanide ISA 51 (Seppic Inc., Fairfield, NJ). The mice received three immunizations 20-days apart, and were bled by tail snips for antibody analyses 20 days after each immunization. The sera of each group were pooled for all antibody analyses. Two mice from each group were euthanized 20 days after each immunization and their pooled spleen cells used for determining cytokine-secreting cells by ELISPOT. For priming experiments, mice received a single immunization of the corresponding peptide and were euthanized 20 days to determine the number of cytokine-secreting cells.

2.2. Synthetic peptides and Linear Peptide Chimeras (LPCs)

The design of LPCs based on promiscuous T cell epitopes reported in *P. vivax* MSP-1 has been previously described [11]. The linear sequences included cysteine residues at the amino and carboxy terminals of the peptides, thus allowing spontaneous polymerization (Fig. 1). Monomer peptides containing the same linear sequences but without cysteine residues were also synthesized (see Fig. 1 and Table 1). *P. berghei* and *P. yoelii* sequences orthologous to the *P. vivax* MSP-1 PvT53 promiscuous T cell epitope [11] and the universal T cell epitope, T*, from the *P. falciparum* circumsporozoite pro-