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Immunology Letters 111 (2007) 92–102
Immunology Letters
www.elsevier.com/locate/

Targeting antigen to MHC Class I and Class II antigen presentation pathways for malaria DNA vaccines

Carlota Dobaño [a,b,*], William O. Rogers [a,1], Kalpana Gowda [a], Denise L. Doolan [a,c,2]

[a] *Malaria Program, Naval Medical Research Center, Silver Spring, MD 20910-7500, United States*
[b] *Henry M. Jackson Foundation, Rockville, MD 20852, United States*
[c] *Department of Molecular Microbiology and Immunology, School of Hygiene and Public Health, Johns Hopkins University, Baltimore, MD 21205-2179, United States*

Received 5 March 2007; received in revised form 4 May 2007; accepted 24 May 2007
Available online 18 June 2007

Abstract

An effective malaria vaccine which protects against all stages of *Plasmodium* infection may need to elicit robust $CD8^+$ and $CD4^+$ T cell and antibody responses. To achieve this, we have investigated strategies designed to improve the immunogenicity of DNA vaccines encoding the *Plasmodium yoelii* pre-erythrocytic stage antigens PyCSP and PyHEP17, by targeting the encoded proteins to the MHC Classes I and II processing and presentation pathways. For enhancement of $CD8^+$ T cell responses, we targeted the antigens for degradation by the ubiquitin (Ub)/proteosome pathway following the N-terminal rule. We constructed plasmids containing PyCSP or PyHEP17 genes fused to the Ub gene: plasmids where the N-terminal antigen residues were mutated from the stabilizing amino acid methionine to destabilizing arginine, plasmids where the C-terminal residues of Ub were mutated from glycine to alanine, and plasmids in which the potential hydrophobic leader sequences of the antigens were deleted. For enhancement of $CD4^+$ T cell and antibody responses, we targeted the antigens for degradation by the endosomal/lysosomal pathway by linking the antigen to the lysosome-associated membrane protein (LAMP). We found that immunization with DNA vaccine encoding PyHEP17 fused to Ub and bearing arginine induced higher IFN-γ, cytotoxic and proliferative T cell responses than unmodified vaccines. However, no effect was seen for PyCSP using the same targeting strategies. Regarding Class II antigen targeting, fusion to LAMP did not enhance antibody responses to either PyHEP17 or PyCSP, and resulted in a marginal increase in lymphoproliferative $CD4^+$ T cell responses. Our data highlight the antigen dependence of immune enhancement strategies that target antigen to the MHC Class I and II pathways for vaccine development.

Keywords: *Plasmodium yoelii*; Malaria; DNA vaccines; PyHEP17; PyCSP; Ubiquitin; LAMP; MHC; Antigen targeting; Antibody responses; T cell responses

1. Introduction

The immunogenicity and protective capacity of plasmid DNA vaccines in animal models has been established in a number of systems, including malaria [1–4]. However, these first generation DNA vaccines are not optimal, and considerable efforts are now being directed at immune enhancement strategies [5–7].

In mice, immunization with DNA vaccines encoding *Plasmodium yoelii* circumsporozoite protein (PyCSP) and *P. yoelii* hepatocyte erythrocyte protein 17 kDa (PyHEP17) confers sterile protection against *P. yoelii* sporozoite challenge [1,2]. This protection is dependent on IFN-γ and $CD8^+$ T cells [1,2] and therefore requires efficient processing and presentation of the DNA-encoded antigen via the MHC Class I pathway [8]. The generation of minimal $CD8^+$ T cell epitopes mostly depends on the degradation of the target antigen by the proteosome complex in the cytoplasm of the antigen-presenting cell (APC) [9]. Attachment of ubiquitin (Ub) [10] to lysine side chains of a protein has been reported to target that protein for rapid cytoplasmic degradation [11,12]. According to the N-end rule [13,14], proteins bearing destabilizing amino acids, such as arginine (R), at the amino terminus are rapidly ubiquitinated and degraded by the proteosome [15,16].

* Corresponding author. Current address: Centre de Recerca en Salut Internacional de Barcelona (CRESIB), Hospital Clínic/Institut d'Investigacions Biomèdicas August Pí i Sunyer (IDIBAPS), Universitat de Barcelona, Barcelona E-08036, Spain. Tel.: +34 932275706; fax: +34 932279853.
E-mail address: cdobano@clinic.ub.es (C. Dobaño).
[1] Current address: Naval Medical Research Unit 2, Jakarta, Indonesia.
[2] Current address: Queensland Institute of Medical Research, The Bancroft Centre, 300 Herston Road, PO Royal Brisbane Hospital, Brisbane, QLD 4029, Australia.

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doi:10.1016/j.imlet.2007.05.007

In addition to efficient antigen processing and presentation via the MHC Class I pathway, effective $CD8^+$ T cell responses are dependent upon the help provided by $CD4^+$ T cells [17,18]. Effective $CD4^+$ T cell help is also required for the induction of high titer specific IgG antibodies, which in the malaria rodent model are associated with protection against blood stage parasitemia [19] and in humans are major contributors to the development of naturally acquired immunity [20]. Nevertheless, current generation DNA vaccines have failed to induce antibodies in the clinic [21–24]. The induction of $CD4^+$ T cell effector responses requires most of the times proteolytic degradation of the antigen via the endosomal/lysosomal processing compartment and presentation in the cell surface by MHC Class II molecules. A family of lysosome-associated membrane proteins (LAMP) located in lysosomes and late endosomes and which contain a C-terminal YQTI sequence has been shown to target proteins through a vesicular pathway to lysosomes [25].

An optimal vaccine to protect against all stages of *Plasmodium* infection would ideally elicit $CD8^+$ and $CD4^+$ T cell and antibody responses and thus need to efficiently deliver antigen to both the MHC Class I and Class II processing and presentation pathways. Accordingly, this study investigates strategies for targeting the *P. yoelii* antigens encoded by DNA vaccines to MHC Class I or II pathways in an attempt to improve their immunogenicity and protective efficacy.

To target the encoded antigen to the MHC Class I pathway we took advantage of the fact that the carboxy terminus of Ub is a substrate for a site-specific protease [26], so that linear fusions of Ub with a protein of interest are cleaved just distal to the last amino acid of Ub. This exposes the first amino acid of the fusion partner, making it possible to engineer proteins which will bear either stabilizing or destabilizing amino acids at the N-terminus following cleavage from the Ub fusion [11,15]. We speculated that ubiquitination should increase the availability of antigenic peptides for binding to MHC Class I molecules and that subsequent recognition by $CD8^+$ T cells should be reflected in the magnitude of Ag-specific $CD8^+$ T cell-mediated immune responses. This strategy has been successful at targeting viral proteins for cytoplasmatic degradation and increased CTL responses in some systems: vaccinia-expressed influenza hemagglutinin [27], HIV envelope protein [28], or HIV nef protein [29]; but not in others: influenza nucleoprotein [30] or Hep C virus core protein [31], expressed in DNA vaccines. Another related strategy has been to take advantage of the fact that alanine (A) at the C-terminus of Ub prevents the cleavage of the Ub-specific protease and provides a substrate for polyubiquitination [32]. Thus, substitution of glycine by A at the C-terminus of Ub fused to a DNA vaccine-encoded protein can result in increased degradation, CTL and protection, as observed in the LCMV nucleoprotein system [33,34].

To target the DNA-encoded Ag to the MHC Class II pathway, we speculated that fusion of the gene of interest to LAMP would target the encoded Ag for processing through the endosomal pathway and presentation via Class II molecules to $CD4^+$ T cells. This approach has resulted in enhanced MHC Class II mediated responses in a number of systems such as HPV-16 E7 [35], HIV envelope protein [36], Epstein–Barr and influenza viruses [37] and CMV protein pp65 [38] but not in the case of HCV core protein [31].

Accordingly, herein, we evaluated the potential of these Ag targeting strategies for the enhancement of vaccine-induced immunity to malaria. Specifically, we compared the immunogenicity induced by plasmid DNA vaccines encoding the pre-erythrocytic antigens PyCSP and PyHEP17 fused to Ub in accordance with the N-end rule, with or without their signal sequence, or fused to LAMP, to responses induced by immunization with unmodified DNA vaccines.

2. Materials and methods

2.1. Plasmid DNA vaccines

Unmodified plasmid DNA vaccines, based on the VR1012 backbone [39], were obtained from Vical Inc. (San Diego, CA): VR2513 encoded PyHEP17 [2], and VR2507 encoded PyCSP [1].

2.2. Cloning of Ub and LAMP fusion constructs

A series of plasmid DNA vaccines encoding PyCSP and PyHEP17, with or without their native signal sequences, fused to the Ub or the LAMP mouse genes, were constructed. Ub fusion constructs were designed to bear either stabilizing or destabilizing amino acids at the amino terminus. This was achieved by mutating the codons encoding the first residue of the PyCSP or PyHEP17 antigen from the stabilizing amino acid M to the destabilizing R. In addition, in plasmids designed to lack the potential hydrophobic leader sequence of the antigen (signal sequence, –ss), the first amino acid distal to the Ub fusion was designed to be either M or R. Another targeting approach, not based on the N-end rule, was to fuse the malaria proteins to a mutant form of Ub with A instead of glycine in the last position (C-terminal). This mutation links Ub to the protein by a covalent bond which is relatively resistant to cleavage, thus targeting the stably ubiquitinated protein into the polyubiquitination pathway and hence to the proteasome. The mouse Ub gene, encoding a 76 amino acid protein highly conserved among eukaryotes [40], was amplified by PCR from genomic DNA. The Ub monomer was gel purified and cloned into pCR-script plasmid (Stratagene, La Jolla, CA). Recombinant PCR, using a series of four primers per reaction, was used to fuse the Ub gene in-frame to the *P. yoelii* genes PyCSP and PyHEP17. Amino acid modifications were introduced with a set of PCR oligonucleotides including the appropriate nucleotides at the junction of the two chimeric genes. Alternatively, site-directed mutagenesis of the relevant amino acids was carried out using the QuikChange™ kit (Stratagene, La Jolla, CA) as described by the manufacturer. PCR products were digested with *Bam*HI, purified by GeneClean II (Bio 101, Q-BIOgene, Morgan Irvine, CA), and ligated to linear dephosphorylated VR1012 plasmid at the *Bam*HI restriction site. In some instances, PCR products were subcloned into pCR-script plasmid prior to cloning into VR1012. DH10β *Escherichia coli*