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Influence of glycosylphosphatidylinositol anchorage on the efficacy of DNA vaccines encoding *Plasmodium yoelii* merozoite surface protein 4/5

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Abstract

Immune responses induced to DNA vaccination vary considerably and depend on a variety of factors, including the physical form in which the antigen is expressed by target cells and presented to the immune system. Data on the effect of these factors will aid improved design of DNA vaccines and facilitate their further development. We examined the effect of different forms of surface anchoring on the immunogenicity of a DNA vaccine. A number of constructs were generated encoding *Plasmodium yoelii* merozoite surface protein 4/5 (PyMSP4/5) with or without its C-terminal glycosylphosphatidylinositol (GPI) attachment signal, replacing the endogenous GPI signal of PyMSP4/5 with that of mouse decay-accelerating factor (DAF), a well-established model for GPI-anchoring in mammalian cells, or the transmembrane anchor and cytoplasmic tail of mouse tissue factor (TF). All constructs were demonstrated to express the full-length PyMSP4/5 in transfected COS cells and induce PyMSP4/5-specific antibodies in mice. The GPI attachment signal of PyMSP4/5 was found to function poorly in mammalian cells and result in a much lower level of PyMSP4/5 expression in vitro than its mammalian counterpart. The DNA vaccine containing the mammalian GPI attachment signal induced the highest levels of antibodies and impacted Ig isotype distribution, consistent with the presence of a CD1-restricted pathway of Ig formation to GPI-anchored membrane proteins. Despite the induction of specific antibodies, none of these DNA vaccines induced sufficient levels of antibodies to protect mice against a lethal challenge with *P. yoelii*.

Keywords: PyMSP4/5; DNA vaccination; Antibody response

1. Introduction

Plasmodium falciparum merozoite surface protein 4 (MSP4) and 5 (MSP5) are two glycosylphosphatidylinositol (GPI)-anchored integral membrane proteins that are potential components of a subunit vaccine against malaria [1,2]. The single homologue (MSP4/5) in the rodent malaria species *Plasmodium yoelii*, *Plasmodium chabaudi* and *Plasmodium berghei* has structural features similar to both MSP4 and MSP5 [3,4], and in *P. yoelii*, PyMSP4/5 has been shown to be highly effective at protecting mice against lethal challenge following immunisation with recombinant protein expressed in *Escherichia coli* [5,6]. Immunisation with DNA vaccines encoding *P. chabaudi* MSP4/5 (PcMSP4/5) has also been shown to provide protection against blood stage infection [7]. MSP4 and MSP5 are now being developed as two components of a multistage, multiantigen malaria vaccine based on DNA immunisation technology [8].

Immunisation with plasmid DNA allows in vivo expression of antigens resulting in the generation of both humoral and cell-mediated immune responses [9]. The qualitative and quantitative aspects of immune responses induced by DNA vaccination have been shown to vary considerably and de-

Abbreviations: MSP, merozoite surface protein; GPI, glycosylphosphatidylinositol; DAF, decay-accelerating factor; TF, tissue factor

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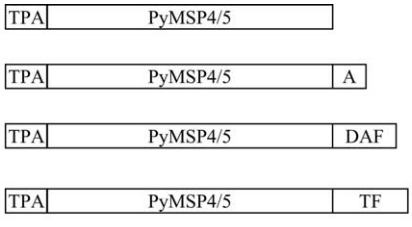
pend on a number of factors, among which the cellular location of the antigen plays an important role. Data on the effect of these factors are useful for the design of DNA vaccines and their further improvement. The presence of an N-terminal secretion signal, either endogenous or heterologous, has been shown to be critical in eliciting a particular type of immune response, as determined by whether the target protein is secreted or remains in the cytoplasm of the transfected host cells [10]. Increased levels of antibodies induced by DNA vaccination with constructs that produce secreted proteins compared to constructs that lead to cytoplasmic expression have been reported for most but not all antigens [10–15]. The presence of a C-terminal transmembrane domain, which results in a membrane-bound protein, has also been reported to influence the immune responses after DNA vaccination. For example, membrane-bound ovalbumin and rabies glycoprotein generate much lower antibody responses than their corresponding secreted forms [10,16], whereas membrane-bound influenza hemagglutinin is more effective than the secreted form [17]. Both forms of the measles hemagglutinin glycoprotein and glycoprotein D from bovine herpesvirus 1 are equally efficacious in inducing antibody [18,19]. Cellular localisation of the expressed antigen also affects the isotype of the antibodies induced by DNA immunisation. Plasmids expressing secreted soluble antigens, when delivered intradermally by gene gun, generally induce a predominantly IgG1 response, whereas plasmids expressing cytoplasmic and membrane-bound antigens tend to induce a more balanced response including IgG2a antibodies [10–12,17,19–22]. It should be noted that the membrane-bound antigens examined in these experiments resulted from anchoring by a transmembrane spanning domain. There has been no systematic study on the effect of type of anchor on the immune responses induced by DNA vaccines and the effect of GPI anchors has not been examined.

A number of *Plasmodium* merozoite surface proteins, including MSP4 and MSP5, are anchored to the cell membrane by a GPI glycolipid covalently attached to the C-terminus of the proteins [1,2]. We have previously constructed three DNA vaccines expressing MSP4 either in the cytoplasm of transfected cells or secreted from cells under the control of the human tissue plasminogen activator (TPA) signal or the native MSP4 signal [23]. We showed that only the construct containing the TPA signal induced detectable antibodies in mice, although gene expression was demonstrated in all constructs and MSP4 was shown to be secreted under either signal by in vitro transient transfection of COS cells [23]. In the present study, we set out to examine the effect of a C-terminal GPI anchor on immune responses induced by DNA vaccination. In order to study the protective efficacy of the antibodies, we utilised the *P. yoelii* challenge model and constructed DNA vaccines encoding PyMSP4/5. We made plasmids that produce secreted soluble PyMSP4/5 or non-secreted PyMSP4/5 containing its GPI signal sequence. Because of the differences between the plasmodial and mammalian GPI signal sequences [24], we replaced the endogenous GPI signal of PyMSP4/5 with that of mouse decay-accelerating factor (DAF), a well-established model for mammalian GPI-anchored proteins [25]. We have also made a plasmid encoding PyMSP4/5 with the transmembrane domain and cytoplasmic tail from mouse tissue factor (TF) fused at its C-terminus to produce a differently anchored PyMSP4/5. We show that the PyMSP4/5 + DAF plasmid induced the strongest antibody responses with elevation of IgG2a.

2. Materials and methods

2.1. Construction of DNA vaccines

Four DNA vaccines were constructed using expression vector VR1020 (Vical Inc., San Diego, CA). All constructs contained the same full-length mature PyMSP4/5 gene cloned downstream of and in frame with the human TPA signal sequence, but with different C-terminal sequences (Fig. 1A). PyMSP4/5 was the entire coding sequence of PyMSP4/5 lacking the N-terminal secretion signal and the C-terminal GPI attachment signal; PyMSP4/5 + A lacked only the N-terminal secretion signal but included its own C-terminal GPI attachment signal; PyMSP4/5 + DAF and PyMSP4/5 + TF contained the same PyMSP4/5 sequence with the GPI-anchoring signal from mouse DAF [26] and the transmembrane domain and cytoplasmic tail from mouse TF [27] attached to the C-terminus respectively. The PyMSP4/5 sequences were amplified by polymerase chain reaction from plasmid pMC481, which contains the full-length PyMSP4/5 cDNA [4]. The 114 bp DNA fragment encoding the final 38 amino acids of mouse DAF and the 135 bp DNA fragment encoding the final 45 amino acids of mouse TF (Fig. 1B) were constructed by ligation of appropriate complementary



(A)

mDAF: STDKGEPNTGGDRYIYGHTCLITLTVLHVMLSLIGYLT

mTF: GETLIIVGAVVLLATIFIILLSISLCKRRKNRAGQKGKNTPSRLA

(B)

Fig. 1. Schematic of the DNA constructs encoding PyMSP4/5 (A) and the amino acid sequences of additional residues that are added at the C-terminus of PyMSP4/5 (B). TPA: human tissue plasminogen activator; PyMSP4/5: full-length mature PyMSP4/5 lacking the N-terminal secretion signal and C-terminal GPI-anchor attachment signal; A: GPI attachment signal of PyMSP4/5; DAF: GPI attachment signal of mouse decay-accelerating factor; TF: transmembrane domain and cytoplasmic tail of mouse tissue factor.