Available online at www.sciencedirect.com
ScienceDirect
Vaccine 25 (2007) 1426–1436
Vaccine
www.elsevier.com/locate/vaccine

Expression and immunogenicity of the *Plasmodium falciparum* circumsporozoite protein: The role of GPI signal sequence

Olga J.A.E Ophorst [a,b], Katarina Radošević [a], Krista Ouwehand [a], Wouter van Beem [a], Ratna Mintardjo [a], Jeroen Sijtsma [a], Jorn Kaspers [a], Arjen Companjen [a], Lennart Holterman [a], Jaap Goudsmit [a,b], Menzo J.E. Havenga [a,*]

[a] *Crucell Holland BV, P.O. Box 2048, Leiden 2301 CA, The Netherlands*
[b] *Center of Poverty-related Communicable Diseases, Academic Medical Center, Amsterdam, The Netherlands*

Received 4 September 2006; received in revised form 23 October 2006; accepted 23 October 2006
Available online 7 November 2006

Abstract

Previous studies have shown that the immunogenicity of rodent malaria parasite-derived circumsporozoite protein (CS) can be improved by deleting the glycosyl-phosphatidyl-inositol (GPI) signal sequence. To study whether GPI signal sequence deletion would also improve immunogenicity of CS derived from the major plasmodium species causing mortality in humans (*P. falciparum*), we tested different variants of the *P. falciparum* CS protein in the context of a live vector-based vaccine carrier (rAd35). We demonstrate that deletion of the GPI signal sequence from CS did not result in altered expression or secretion. In contrast, cellular localization was clearly altered, which perhaps helps to explain the significant improvement of anti-CS antibody and T-cell responses observed in mice using deletion variants in the context of the rAd35 carrier. Our results show that rational design of antigens is warranted for further development of malaria vaccines.

Keywords: Adenovirus vaccines; Malaria; Circumsporozoite protein; Immunogenicity

1. Introduction

Previously we have reported on the potency of a human recombinant adenovirus type 35 (rAd35)-based vaccine expressing *Plasmodium yoelii* circumsporozoite (CS) antigen. We demonstrated induction of both CS-specific T-cells and antibodies in mice, as well as protection upon high-dose sporozoite challenge [1]. The rAd35 carrier was chosen based on: (i) accumulating data demonstrating that a T-cell response, which is generally induced efficiently by adenovirus [2–6], plays an important role in controlling the malaria parasite [7–9]; and (ii) adenovirus sero-prevalence studies demonstrating that human type 35 is rarely being neutralized with human sera derived from volunteers living in malaria endemic regions [1]. This contrasts with the most commonly used adenoviral vector, rAd5 [10–12].

The *P. yoelii* CS protein was chosen because vaccination with irradiated sporozoites [13–15], as well as studies in the field [16–23], have demonstrated that CS derived from human *Plasmodium* spp. represents an important immunological target. High and frequent immune responses against this protein have been established in humans.

The *P. yoelii* CS protein used in our earlier studies contained CS protein with partially deleted GPI signal sequence [24]. This followed studies in established mouse malaria models, which had shown that presence of the complete GPI signal sequence in *P. berghei* and *P. yoelii* CS protein led to impaired anti-CS immune responses [25,26].

Subsequent studies have suggested that presence of the GPI signal sequence sequentially affected total CS protein production, cellular distribution, antigen processing and secretion, leading to less effective antigen presentation [26].

* Corresponding author. Tel.: +31 71 5248736; fax: +31 71 5248702.
E-mail address: m.havenga@Crucell.com (M.J.E. Havenga).

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doi:10.1016/j.vaccine.2006.10.050

The GPI signal sequence is known to serve as a recognition site for transamidases localized in the endoplasmic reticulum (ER), which upon recognition cleaves a C-terminal propeptide from the CS proprotein. Subsequently, a GPI anchor is provided and the CS protein is transported and expressed on the cell surface of the *Plasmodium* spp. [27–29]. However, the transamidase reaction is highly species-specific. Studies have shown that parasitic proteins with GPI signal sequence that remain non-cleaved stick in the internal cell organelles of mammalian cells [30]. For the *P. falciparum* species, which causes high mortality in humans it remains to be investigated whether presence or absence of the GPI signal sequence in *P. falciparum* CS protein influences the expression, secretion and antigen presentation.

Therefore, we designed CS proteins carrying two different GPI deletion mutants: one carrying a complete deletion of the GPI signal sequence (Δ28) and one CS protein carrying a 14 amino acid deletion, thereby deleting only the hydrophobic sequence at the C-terminus [31]. The role of the GPI signal sequence was assessed in the context of the rAd35 carrier, since this allows immediate selection of the most immunogenic *P. falciparum* CS sequence in the context of a potent vaccine. The data obtained demonstrate that deletion of the GPI signal sequence from the *P. falciparum* results in increases in both B- and T-cell responses, as compared to full length CS protein.

2. Material and methods

2.1. Adenovirus production and cell transduction

Replication-incompetent Ad35 vectors were generated in PER.C6/55 K cells using pBR322-based adaptor plasmid pAdApt535 together with cosmid pWE.Ad35.AflIII-rITRΔE3 as previously described [32]. The adaptor plasmid contained the left portion of the Ad genomes nt 1–464 followed by transcriptional control elements and the adaptor Ad DNA region, nt 3401–4669. The circumsporozoite consensus sequence is gathered by the alignment of different available protein sequences present in the GeneBank database. All CS complete or partial sequences were used in order to identify variation between the different geographical areas and identified laboratory strains to determine the final consensus sequence. The sequence, representing the full length CS protein was optimized for high levels of expression in mammalian cells (GeneArt, Regensburg, Germany) and cloned into the expression cassette in the adaptor plasmid. The resulting pAdApt535-CS plasmid expressed full length *Plasmodium falciparum* CS under transcriptional control of the human full length immediate-early CMV promotor and the SV40 polyadenylation signal. CS GPI signal sequences deletion mutants were amplified by the polymerase chain reaction (PCR) using pAdApt535-CS plasmid as template. A PCR fragment encoding for aa 1–358 of CS, deleted for the GPI signal sequence was amplified with a Forw Falc.CS 5′-CCAAGCTTGCCACCATGATGAGG-3′ (sense) and anti-sense primer Rev Falc.CS-28 5′-CCGGATCCTCAGCAGATC-TTCTTCTCG-3.

The CS sequence partial deleted for the GPI signal (aa 1–372) was amplified with the Forw Falc.CS primer as described above and Rev.Falc.CS-14 5′-CGGATCCTCA-GCTG-TTCACCACGTTG-3, respectively. The amplified PCR products were digested with the restriction enzymes *Hin*dIII and *Bam*HI and cloned into pAdApt535 and co-transfected into PER.C6/55 K® cells together with the cosmid pWE.Ad35.AflIII-rITRΔE3.

All generated recombinant Ad35 vectors were purified by cesium chloride density centrifugation and vaccine preparations were stored at −80° C until further use. The virus titer, expressed as the number of virus particles (vp) per milliliter, was determined by high-performance liquid chromatography. Quality control testing of virus batches included identity PCR, absence of mycoplasma, remaining cesium chloride content, bioburden and vp/plaque-forming unit ratio determination.

Cultured human lung carcinoma A549 cells (ATCC) were exposed to 2500 vp/cell rAd35 expressing Pf.CS antigens of different lengths; full length (rAd35Pf.CS FL), deletion of 14 amino acids (rAd35Pf.CS Δ14) or 28 amino acids (rAd35Pf.CS Δ28) at the carboxyl terminal, respectively. Cells were cultured in DMEM culture medium supplemented with 10% (v/v) heat inactivated fetal bovine serum and 1× Penicillin/Streptomycin (Gibco BRL) at 37° C and 10% CO_2. Every 24 h samples were harvested for in vitro analysis.

2.2. Western blotting detection of CS and PARP proteins

Human lung carcinoma A549 cells (10^5) were transduced with 2500 vp/cell rAd35 containing Pf.CS antigens or left untreated (control). Cell lysates and corresponding supernatants were harvested and expression levels of proteins were determined by Western blot analysis. Rabbit polyclonal antibodies against Pf.CS (MRA-24, MR4/ATCC) were used in combination with goat anti-rabbit, IgG, conjugated to horseradish peroxide (HRP) (Southern Biotechnology Associates) to detect CS expression. Human GAPDH, detected by an anti-GAPDH antibody (Santa Cruz Biotechnology) was used as loading control. For the detection of apoptosis marker poly-(adenosine diphosphate-ribose)polymerase (PARP) ∼116 kDa protein and ∼85 kDa (apoptosis-induced cleavage fragment), mouse anti-PARP antibody (Biomol Int.) was used in combination with rabbit-anti-mouse antibody conjugated to HRP (Dako cytomation). Neuroblastoma cells (IMR32) treated with trichostatin, a deacetylase inhibitor that induces high levels of apoptosis in these cells, were included as a positive control for apoptosis detection [33].

For all blots the detection was performed by the enhanced chemiluminescence assay (ECL$^+$, Amersham Pharmacia Biotech) according to the manufacturer's instructions.