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Evaluation of a prime-boost vaccine schedule with distinct adenovirus vectors against malaria in rhesus monkeys

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ABSTRACT

A vaccine that elicits both specific antibodies and IFN-γ-producing T cells is required to protect against pre-erythrocytic malaria. Among the most promising approaches to induce such complex immunity are heterologous prime-boost vaccination regimens, in particular ones containing live viral vector. We have demonstrated previously that adenovectors serotype 35 (Ads35) encoding the circumsporozoite (CS) antigen or liver-stage antigen-1 (LSA-1) are highly effective in improving the T-cell responses induced by immunizations with protein-based vaccines in a heterologous prime-boost schedule. Here we evaluated the potential of a heterologous prime-boost vaccination that combines the Ad35.CS vector with the serologically distinct adenovector Ad5.CS, in rhesus macaques, after establishing the potency in mice. We show that the heterologous Ad35.CS/Ad5.CS prime-boost regimen elicits both antibody responses and robust IFN-γ-producing $CD8^+$ T-cell responses against the CS antigen. Analysis of the quality of the antibody responses in rhesus macaques, using indirect immunofluorescence assay (IFA) with *Plasmodium falciparum*-coated slides, demonstrated that this heterologous prime-boost regimen elicits a high titer of antibodies that are able to bind to *P. falciparum* sporozoites. Level of the IFA response was superior to the response measured with sera of an adult human population living in endemic malaria region. In conclusion, the combination of Ad35.CS, a vaccine based on a rare serotype adenovirus, with Ad5.CS or possibly another adenovector of a distinct serotype, induces a complex immune response that is required for protection against malaria, and is thus a highly promising approach for pediatric vaccination.

1. Introduction

Every year malaria kills one million people, from which a large majority are children under 5 years of age [1]. Pediatric vaccination against malaria is thus desperately needed. Although the feasibility of a malaria vaccine was demonstrated using irradiated sporozoites more than 30 years ago [2], an effective malaria vaccine has not been generated as yet. The most advanced of the malaria vaccine candidates currently being tested in human clinical trials is the pre-erythrocytic circumsporozoite (CS)-protein-based vaccine RTS,S, formulated with AS adjuvants. Albeit this vaccine demonstrated only a limited protection in field trials [3–7], a large phase III trial has been initiated and, provided that positive results are achieved, the licensure of the vaccine is expected in a few years. The limited protection induced with RTS,S is probably due to the vaccine failure to induce $CD8^+$ T-cell responses while eliciting strong humoral immunity, a feature attributable to protein-based vaccines in general [8,9]. The results obtained with RTS,S support the feasibility of a pre-erythrocytic vaccine based on the CS antigen, but also reinforce the vision that an efficacious vaccination against the liver-stage malaria will unquestionably require induction of both antibody responses and cellular immunity.

Heterologous prime-boost vaccinations involving viral vectors, specifically designed to elicit both antibody and T-cell responses, are being evaluated for malaria [10–13]. Rationale for this approach is based on the notion that different types of vaccines engage different antigen-processing pathways, with heterologous combinations efficiently stimulating multiple components of the immune system. Among the heterologous prime-boost combinations, schedules involving adenoviral vectors are particularly interesting. The appealing attributes of adenovectors for vaccination lay in their safety and their ability to infect a broad range of both actively

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dividing as well as non-dividing mammalian cells, to express high levels of the genes incorporated and to induce potent antibody and cellular responses when administered by different immunization routes [14–17]. An additional important advantage is the availability of a mature industrial manufacturing platform based on the PER.C6® cell line, which enables cost-effective production of sufficient vaccine dosages for mass vaccination [18,19]. Adenovectors have proven successful when combined in heterologous prime-boost regimens with other type of vaccines, such as DNA [20–22], poxvirus vectors [23,24] or BCG [25,26]. In recent animal studies we have demonstrated the advantage of combining adenovectors with protein-based vaccines. In a non-human primate study, immunizations combining the adenovector serotype 35 expressing the CS antigen (Ad35.CS) and RTS,S were highly effective in eliciting strong and sustainable T-cell as well as antibody responses [10]. The superiority of the heterologous combination with Ad35 and protein versus homologous counterparts has been corroborated in a mouse study with another malarial antigen, the liver-stage antigen-1 (LSA-1) [11].

Recently, promising heterologous prime-boost regimens that combine SIV antigen-expressing adenovectors of different serotypes have been described, demonstrating an advantage of the heterologous combination in eliciting a potent T-cell immunity with a broad polyfunctional phenotype and a protection in a stringent rhesus macaques challenge model [27].

In the current study, we have evaluated the ability of a heterologous prime-boost schedule using two serologically distinct adenovectors, Ad35 (B type) and Ad5 (C type), expressing *Plasmodium falciparum* CS antigen to elicit the humoral and cellular immune responses that are required for protection against malaria.

2. Materials and methods

2.1. Adenoviral vectors and immunizations

The E1/E3-deleted rAd35 and rAd5 adenovectors encoding for the *P. falciparum* CS antigen were generated using a procedure described elsewhere [19,28,29]. The CS gene is a synthetic codon-optimized insert encoding a CS protein based on the EMBL protein sequence CAH04007, and truncated for the last 14 amino acids at the C-terminus [30]. The N-terminus of this CS protein is a consensus assembled by alignment of various sequences present in the GenBank, while the repeat region and the C-terminus is based on the sequence of the 3D7 *P. falciparum* clone.

The potency of the heterologous Ad35.CS/Ad5.CS prime-boost regimen was first determined in mice, followed by evaluation of immunogenicity in rhesus macaques. For the mouse studies, BALB/c female mice, 6–8 weeks old, were purchased from Harlan (Zeist, The Netherlands) and kept at the Animal Facility of Crucell Holland B.V. under specified pathogen-free conditions. Institutional Committees for Animal Care and Use reviewed and approved the mouse experiments. The heterologous prime-boost regimen in mice consisted of a prime immunization with Ad35.CS followed by a boost with Ad5.CS (prime-boost group, eight mice). As control for boosting, a group of mice received a prime with Ad35.CS followed by an immunization with an empty (without the transgene) adenovector (AdE) (prime-only group, eight mice). An additional group of mice received two immunizations with the corresponding empty adenovectors (negative control group, 4 mice). A homologous prime-boost control group, receiving two subsequent immunizations with the Ad35.CS vector, would have been ideal as comparator group for the heterologous regimen. However, as published in earlier studies such type of homologous prime-boost immunization regimen is not feasible in mice due to the high levels of adenovector neutralizing antibodies that are induced upon the prime immunization, which hamper the effect of the boost immunization [29,31,32]. Mice were intramuscularly (IM) immunized, at 0 and 4 weeks, with 10^9 vp of the adenovectors prepared in a total volume of 100 μl of PBS containing 5% sucrose (50 μl/quadriceps of hind legs). Two weeks after the last immunization sera and spleen cells were collected for determination of the CS-specific antibody and T-cell responses.

For evaluation of immunogenicity of heterologous Ad35.CS/Ad5.CS prime-boost regimen in the non-human primates, rhesus macaques (6 animals) were selected from an available animal pool based on following criteria: (i) physical exam demonstrating a good health, (ii) no prior exposure to malaria and malaria antigens (no background in CS-specific immunological assay), and (iii) no detectable neutralizing antibodies against Ad35 or Ad5 in serum. All animals were maintained in accordance with the *Guide for the Care and Use of Laboratory Animals* [33], and with the approval of the Institutional Animal Care and Use Committee of Harvard Medical School. Animals were housed in quarantine for 5 weeks, to allow complete acclimatization of animals prior to vaccination. Rhesus macaques were immunized twice, at 0 and 12 weeks, with 10^{11} vp of Ad35.CS and at 25 weeks with 10^{11} vp of Ad5.CS. The adenovectors were prepared in 0.5 ml of PBS containing 5% sucrose. Upon immunizations, animals were monitored by relevantly trained personnel for vaccination-related adverse effects, including monitoring of behaviour and body weight (daily for 1 week after each vaccination), hematology and clinical chemistry analysis (at 1, 3, 7 and 14 days after each vaccination). Adverse effects were neither observed after Ad35.CS nor after Ad5.CS vaccination (*data not shown*). At defined time points upon immunizations, serum and peripheral blood mononuclear cells (PBMC) were collected for evaluation of the CS-specific antibody and T-cell responses and the neutralizing antibodies against the adenovectors.

2.2. Human serum samples

Liberian human serum samples from healthy adults (>15 years) living in a *P. falciparum* malaria holoendemic and perennial area were kindly provided by Professor Marita Troye-Blomberg (Stockholm University, Sweden).

2.3. Neutralization assays

The neutralizing antibodies induced against the adenovectors were determine using neutralizations assays for Ad35 and Ad5 adenovectors as previously described [34]. Briefly, 1/32 diluted sera were added to 96-wells flat-bottom microtiter plates (Greiner Bio-one, Alpen a/d Rijn, The Netherlands) and serially two-fold-diluted. Recombinant adenovirus containing the luciferase reporter gene (rAd35.Luc, rAd5.Luc) and A549 cells were added to the plates. The luciferase reporter gene expression in the A549 cells was measured using luciferase substrate (PerkinElmer, Waltham MA) and a Trilux luminescence detector (according to the manufacturer's instructions). Data was analyzed using non-linear regression to calculate the antibody inhibitory concentration of 90% (IC_{90}) in the sera samples.

2.4. CS-specific ELISA

The CS-specific antibody responses were measured using ELISA. The ELISA procedure used for the mouse samples was performed as described elsewhere [35]. Ninety-six-well microtiter plates (Maxisorp, Nunc) were coated overnight at 4 °C with 2 μg/ml of CS specific peptide $(NANP)_6C$ in 0.05 M carbonate buffer. After washing the plates, 1:100 diluted individual serum samples were added to the wells and serially two-fold-diluted. Plates were incubated for