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Vaccine 23 (2005) 1451–1456
Vaccine
www.elsevier.com/locate/vaccine

Characterization of the immune response to DNA vaccination strategies for schistosomiasis candidate antigen, Sm-p80 in the baboon

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Received 2 July 2004; received in revised form 27 August 2004; accepted 28 September 2004
Available online 30 October 2004

Abstract

Even though schistosomicidal agents and other control measures, including public hygiene and snail control exist, development of an efficacious vaccine still remains the most potentially powerful method for control of schistosomiasis. In our continuing efforts to develop a vaccine against schistosomiasis, we have selected a vaccine candidate (Sm-p80), which plays an important role in the immune evasion process of the parasite. Sm-p80 has been shown to confer up to 60% protection in mice following experimental infection. In this initial study, we have used Sm-p80 plus the Th1 response promoting cytokine, interleukin-2 (IL-2), in a DNA immunogen formulation. The vaccine was tested for its safety and immunogenicity in a baboon model of schistosomiasis. The vaccine generated a Th1 type Sm-p80-specific response in baboons with IgG_1/IgG_2 ratios of less than 1.0. No detectable IgG_3 or IgG_4 anti-Sm-p80 responses were present in the immunized baboons. The antibodies to Sm-p80 were able to kill up to 35% schistosomula in vitro in the presence of complement. These results although preliminary suggest the potential of Sm-p80 as a viable vaccine candidate for schistosomiasis.

Keywords: DNA vaccination; Schistosomiasis; Sm-p80; Calpain

1. Introduction

During the last 25 years, many investigators have attempted to identify schistosome antigens that induce partially protective immune responses. More than 100 such antigens have been identified, about 15% of which provide varying degrees of protection and are considered promising [1]. It is important to note that none of these candidate antigens have induced levels of an immune response approaching to sterile immunity (~80% protection) that has been observed following vaccination with irradiated schistosome larvae [1]. Furthermore, two laboratories with recognized experience in schistosomiasis, independently and under the World Health Organization (WHO) delineated immunization and challenge regimen, assessed the protective potential of the six "priority antigens" (paramyosin, glutathione *S*-transferase, fatty acid binding 14 kDa protein, IrV-5, triose phosphate isomerase and Sm23) [1,2]. None of the six antigens in these studies provided stated goal of 40% protection or better, in the mouse model [1,2]. In spite of this, some of these antigens have been slated to be developed for human trials [3]. One of these antigens, *Schistosoma haematobium* glutathione *S*-transferase, has already been advanced to clinical trials [4,5], and is now in phase II trials [6]. However, none of the results from phase I trials have been published [7]. Taken this together, it would be prudent to start the careful examination and reassessment of those vaccine candidates that are identi-

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doi:10.1016/j.vaccine.2004.09.018

fied by novel means, play essential role(s) in the survival of the parasite and are easily accessible to the host. One such schistosome antigen is the large subunit of calpain (Sm-p80). This schistosome specific protein was originally identified to be involved in membrane turnover of the surface epithelial syncytium [8]. This phenomenon has been considered to be one of the immune evasion mechanisms utilized by blood-dwelling helminths [8–10].

Using various immunization regimens (DNA and protein) with Sm-p80, four "research groups" have independently observed protection levels ranging from 30 to 60% in mice [11–18]. Based on the WHO recommendations (>50% protection in mice), Sm-p80 is being considered for human phase I trials. However, we believe that Sm-p80 or any other schistosome antigen should not be rushed into clinical trials only on the basis of the protection obtained in the murine model; exhaustive protective studies should also be performed in at least one other animal model, preferably a non-human primate before embarking onto human studies. Therefore, in this pre-clinical pilot study, we have examined the toxicity, safety and antigenicity of Sm-p80 in a non-human primate model of schistosomiasis. Baboons (*Papio* spp.) are natural hosts for schistosomes [19], have been shown to complete the parasite life cycle in the absence of infected humans [20]. This non-human primate model has been utilized as experimental infection and disease models for human schistosomiasis [21–22].

In this report, we demonstrate the type of immune response elicited by the DNA vaccine formulation containing Sm-p80 and IL-2 as well as the killing of schistosomula in vitro in the presence of exogenous complement and antibodies obtained from immunized baboons.

2. Materials and methods

2.1. Immunization protocol and sera collection

In this IACUC-approved study, baboons (*Papio anubis*) were first screened for parasites and for antibodies that were cross-reactive to Sm-p80. Baboons were obtained from the University of Oklahoma Health Sciences Center baboon breeder program and housed at the AAALAC-accrediated Animal Resource Facility. Three baboons per group were immunized, as follows:

Group 1: The initial immunization was with 500 μg of the control plasmid DNA, pcDNA3 (prepared in PBS) [23]. The DNA was injected intramuscularly in the quadriceps. Baboons were boosted with 500 μg pcDNA3 at weeks 4, 8, and 12.

Group 2: The initial immunization was with 500 μg Sm-p80-pcDNA3 (prepared in PBS) [23]. The DNA was injected intramuscularly in the quadriceps. Baboons were boosted with 500 μg Sm-p80-pcDNA3 at weeks 4, 8, and 12.

Group 3: The initial immunization was with 500 μg Sm-p80-pcDNA3 and 500 μg pORF-hIL-2 (prepared in PBS) [23]. The DNA was injected intramuscularly in the quadriceps. Baboons were boosted with 500 μg Sm-p80-pcDNA3 and 500 μg pORF-hIL-2 at weeks 4, 8, and 12.

Blood was collected just prior to the primary immunization, at every booster (i.e., 4, 8 and 12 weeks) and 4 weeks after the final immunization (16 weeks). Baboons were anesthetized with ketamine/xyaline, prior to the venipuncture and then were bled from the femoral vein into vacuum tubes to obtain the sera.

2.2. ELISA

Total IgG, IgG_1, IgG_2, IgG_3 and IgG_4 antibodies were determined by ELISA as described elsewhere [24]. Briefly, 96 well plates were coated with baculovirus generated recombinant Sm-p80 (1.2 μg/well) and blocked with PBS containing 10% FCS. Sera from individual baboons (1:200 dilutions) were added to triplicate wells. As secondary antibodies, horse radish peroxidase labeled anti-human IgG, IgG_1, IgG_2, IgG_3 and IgG_4 [24], were used at a dilution of 1:500. The substrate solution (TMB) was then added to each well and reaction was stopped by 2 M H_2SO_4. The absorbance value was measured at 450 nm. Values obtained from the control group (pcDNA3 group) were subtracted from the experimental groups. Results are expressed as the mean ± S.E. of absorbance from two different experiments (triplicate wells/ELISA). Endpoint titers for Sm-p80 specific IgG were calculated from a binding curve of optical density verses serum (wk 16 bleed) dilution to a cutoff of two standard deviations above background control values.

2.3. Complement-dependent killing of schistosomula in vitro

Two hundred mechanically transformed schistosomula were pre-treated for 30 min in 50 μl RPMI 1640 medium with an equal volume of 1:2 diluted antiserum from individual baboons (from "Sm-p80-pcDNA3 group" or "Sm-p80-pcDNA3 plus pORF-hIL-2 group"). The incubation was carried out at 37 °C in 5% CO_2 atmosphere. As a source of complement fresh guinea-pig serum was then added at 1:9 final dilution, with heat-inactivated complement serving as a control. The resultant cultures were then incubated overnight, following which live and dead parasites were counted [25,26]. Sera from baboons immunized with pcDNA3 alone were used as a negative control. The efficiency of killing was assessed by the difference between the percentage of dead schistosomula in the presence of active and inactivated complement.

2.4. Statistical analysis

Significance between two groups was calculated using the two-sided *t*-test and/or Wilcoxon rank-sum test. *P*-values ob-