International Journal for Parasitology xxx (2018) xxx–xxx

Contents lists available at ScienceDirect

International Journal for Parasitology

journal homepage: www.elsevier.com/locate/ijpara

Prospects of developing a prophylactic vaccine against human lymphatic filariasis – evaluation of protection in non-human primates

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ARTICLE INFO

Article history:
Received 26 January 2018
Received in revised form 29 March 2018
Accepted 4 April 2018
Available online xxxx

Keywords:
Lymphatic filariasis
Vaccine
Non-human primates
Adjuvant
TLR-4 agonist
Multivalent vaccine

ABSTRACT

Lymphatic filariasis (LF) affects 120 million people around the world and another 856 million people are at risk of acquiring the infection. Mass Drug Administration (MDA) spearheaded by the World Health Organization is the only current strategy to control this infection. Recent reports suggest that despite several rounds of MDA, elimination has not been achieved and there is a need for more stringent control strategies for control of LF. An effective prophylactic vaccine combined with MDA has significant potential. Initial trials using a prophylactic trivalent recombinant *Brugia malayi* heat shock protein 12.6, abundant larval transcript 2 and tetraspanin large extra cellular loop (r*Bm*HAT) vaccine developed in our laboratory conferred only 35% protection in macaques. Therefore, the focus of the present study was to improve the current vaccine formulation to obtain better protection in non-human primates. We made two modifications to the current formulation: (i) the addition of another antigen, thioredoxin peroxide (TPX-2) to make it a tetravalent vaccine (r*Bm*HAXT) and (ii) the inclusion of an adjuvant; AL019 (alum plus glucopyranosyl lipid adjuvant-stable emulsion) that is known to promote a balanced Th1/Th2 response. A double-blinded vaccination trial was performed with 40 macaques that were divided into three treatment groups and one control group (n = 10/group). Vaccinated animals received 4 immunisations at 1 month intervals with 150 μg/ml of r*Bm*HAT plus alum, r*Bm*HAT plus AL019 or r*Bm*HAXT plus AL019. Control animals received AL019 only. All vaccinated macaques developed significant ($P \leq 0.003$) titers of antigen-specific IgG antibodies (1:20,000) compared with the controls. One month after the last dose, all macaques were challenged s.c. with 130–180 *B. malayi* L3s. Our results showed that seven out of 10 (70%) of macaques given the improved r*Bm*HAXT vaccine did not develop the infection compared with AL019 controls, of which seven out of 10 macaques developed the infection. Titers of antigen-specific IgG1 and IgG2 antibodies were significantly ($P \leq 0.01$) higher in vaccinated animals and there was an increase in the percentage of IL-4 and IFN-γ secreting antigen-responding memory T cells. These studies demonstrated that the improved formulation (r*Bm*HAXT plus AL019) is a promising vaccine candidate against human lymphatic filariasis.

1. Introduction

Lymphatic filariasis (LF) is a chronic tropical filarial parasitic infection caused by *Wuchereria bancrofti*, *Brugia malayi* and *Brugia timori* and is transmitted by mosquitoes. The disease is characterised by severe physical disability and morbidity in infected individuals (Brady and Global Alliance to Eliminate Lymphatic Filariasis, 2014). Significant progress has been made in the last decade to interrupt the transmission of the disease by administering a selected combination of three drugs annually to all the individuals living in an endemic area (mass drug administration, MDA) (Brady and Global Alliance to Eliminate Lymphatic Filariasis, 2014; Ramaiah and Ottesen, 2014; Bhattacharjee, 2016). Although this MDA approach is highly effective in reducing the transmission of LF infection in most countries, there are several reports of non-compliance by the person being treated, leading to reemergence of the disease in a few parts of the world (Das et al., 2002; Anil, 2012; Lima et al., 2012; Nujum et al., 2012; Krentel et al., 2013; Sunish et al., 2014; Bhattacharjee, 2016; NVBDCP, 2016; WHO,

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https://doi.org/10.1016/j.ijpara.2018.04.002
0020-7519/

2016; Dyson et al., 2017). These findings brought to light the critical need for a more sustainable approach such as a prophylactic vaccine together with MDA to interrupt the transmission and control of LF infection in endemic areas (Kalyanasundaram, 2016). Our laboratory and others have identified and characterised several potential candidate vaccine antigens of LF and evaluated their vaccine potential in rodent models (Denham, 1980; Dissanayake et al., 1995; Gregory et al., 1997; Anand et al., 2008, 2011; Gnanasekar et al., 2008; Vedi et al., 2008; Veerapathran et al., 2009; Kalyanasundaram and Balumuri, 2011; Babayan et al., 2012; Dakshinamoorthy et al., 2012; Anugraha et al., 2013; Dakshinamoorthy et al., 2013a; Gomase et al., 2013; Arumugam et al., 2014; Gupta et al., 2016). Among the various antigens that we characterised, four antigens, abundant larval transcript-2 (ALT-2) (Anand et al., 2008; Kalyanasundaram and Balumuri, 2011; Madhumathi et al., 2017), heat shock protein (HSP) 12.6 (Dakshinamoorthy et al., 2012), thioredoxin peroxidase-2 (TPX-2) (Anand et al., 2008; Anugraha et al., 2013) and tetraspanin large extracellular loop (TSP-LEL) (Gnanasekar et al., 2008; Dakshinamoorthy et al., 2013a) gave excellent protection in rodent models. Subsequently, we showed that combining three of these antigens as a multivalent fusion protein, r*Bm*HAT (recombinant *B. malayi* HSP12.6, ALT-2 and TSP-LEL) gave close to sterile immunity in mouse and jird models (Dakshinamoorthy and Kalyanasundaram, 2013; Dakshinamoorthy et al., 2013a). Based on these promising results in rodents, we performed a vaccination trial in rhesus macaques with r*Bm*HAT and alum adjuvant (Dakshinamoorthy et al., 2014). Unfortunately, however, we only obtained approximately 35% protection against challenge infections in macaques and the immune response elicited was predominantly IgG1/IL-10 driven due to the alum adjuvant. Subsequent vaccination trials with AL019 in a mouse model showed that AL019 (alum plus GLA, a synthetic TLR4 agonist) is a better adjuvant for r*Bm*HAT than alum (Dakshinamoorthy and Kalyanasundaram, 2013; Chauhan et al., 2017). Protective responses in humans and rodents correlated with a balanced Th1/Th2 response and AL019 was shown to promote a balanced Th1/Th2 response (Dakshinamoorthy and Kalyanasundaram, 2013; Dakshinamoorthy et al., 2013a). Therefore, we decided to evaluate the potential of AL019 as an adjuvant, for vaccination in rhesus macaque in this study. In an attempt to improve the vaccine antigen formulation, we included TPX2 as the fourth antigen to the trivalent r*Bm*HAT to make a tetravalent (r*Bm*HAXT) vaccine construct. Thus, the major aim of this study was to evaluate the vaccine potential of r*Bm*HAXT together with AL019 in the rhesus macaque model, to determine whether the improved vaccine formulation gave better protection in rhesus macaques against challenge infections with *B. malayi* infective larvae and assess the immunological correlates of protection.

2. Materials and methods

2.1. Ethics statement

Use of macaques and the experimental procedures performed in this study were reviewed and approved by the The Institutional Animal Care and Use Committee (IACUC) committee at Bioqual Inc, Rockville, MA, USA and by the University of Illinois College of Medicine at Rockford, USA. Humane use of animals was performed in this study according to the guidelines for the care and use of laboratory animals and with the rules formulated under the Animal Welfare Act by the U.S. Department of Agriculture.

2.2. Non-human primates

Forty male or female disease-free rhesus macaques (3–5 years old) were purchased from PrimGen (Hines, IL, USA) and housed at the facility of Bioqual at Rockville, MD, USA. All the procedures for maintenance of the animals were as described previously (Dakshinamoorthy et al., 2013a). All animals were screened for the absence of filarial infections prior to enrolling them in the study by analysing the blood for the presence of microfilarial *Hha-1* by PCR (Hoti et al., 2003; Rao et al., 2006); and serum for the presence of antibodies against r*Bm*SXP-1 (Vasuki et al., 2003; Abdul Rahman et al., 2007), and r*Bm*HAXT proteins were analyzed using an ELISA. Animals that were positive for any of the proteins were not enrolled in the study.

2.3. Parasites

Brugia malayi infective L3s were obtained from the National Institute of Allergy and Infectious Diseases/National Institute of Health (NIAID/NIH), USA, Filariasis Research Reagent Resource Center (University of Georgia, Athens, GA, USA) under an NIAID supply contract AI#30022.

2.4. Adjuvants

Two different adjuvants were compared in this study. Alum (AL007) and Alum plus a synthetic TLR4 agonist GLA (AL019) purchased from the Infectious Disease Research Institute, Seattle, WA, USA.

2.5. Cloning and expression of multivalent recombinant proteins

r*Bm*HAT protein was expressed in the *Escherichia coli* BL21 strain (DE3), purified and analyzed as described previously (Dakshinamoorthy et al., 2014). The coding sequence (CDS) of multivalent fusion protein r*Bm*HAT (consisting of *bmhsp 12.6*, *bmalt-2* and *bmtsp*) and r*Bm*HAXT (consisting of *bmhsp 12.6*, *bmalt-2*, *bmtpx2* and *bmtsp*) were synthesised at GenScript (Piscataway, NJ, USA). The sequences were provided in a pUC 51 vector. Both CDS were PCR amplified using the same gene-specific primers (Forward primer: 5′ CG**GGATCC**ATGGAAGAAAAGGTAGTG 3′ & Reverse primer: 5′ CG**GAATTC**TCAATCTTTTTGAGATGAAT 3′) with restriction sites for *Bam*HI and *Eco*RI, and cloned into the expression vector pRSETA (Invitrogen, Carlsbad, CA, USA) with the 6× Histidine tag. The ligated constructs for both *bmhat* and *bmhaxt* were further transformed into the expression strain of *E. coli* BL21 (DE3). Expression of recombinant proteins was induced with 1 mM Isopropyl β-D-1-thiogalactopyranoside (IPTG). The recombinant proteins were purified using Nickel affinity column chromatography (GE Healthcare Life Sciences, Pittsburg, PA, USA) and the purity of the recombinant proteins was confirmed in a 12% SDS PAGE gel and by western blot using anti-penta His antibodies (Qiagen, Velencia, CA, USA). Endotoxin in the final purified recombinant protein was removed using an endotoxin removal column (Thermo Fisher Scientific, Rockford, IL, USA). The final concentrations of r*Bm*HAT and r*Bm*HAXT proteins were determined by Bradford assays (BioRad Laboratories, Hercules, CA, USA).

2.6. Immunisation of macaques

This was a double-blinded vaccination trial. A total of 40 macaques were randomly divided into three treatment groups and one control group with 10 macaques per group. All the treated animals received four doses of 150 μg of the vaccine antigen and 2 mg of the adjuvant on days 0, 28, 56 and 84. Treatment group 1 received

Please cite this article in press as: Khatri, V., et al. Prospects of developing a prophylactic vaccine against human lymphatic filariasis – evaluation of protection in non-human primates. Int. J. Parasitol. (2018), https://doi.org/10.1016/j.ijpara.2018.04.002