Comparative Immunology, Microbiology and Infectious Diseases 36 (2013) 507–519

Contents lists available at SciVerse ScienceDirect

Comparative Immunology, Microbiology and Infectious Diseases

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

Immunization with a multisubunit vaccine considerably reduces establishment of infective larvae in a rodent model of *Brugia malayi*

Nidhi Shrivastava [1], Prashant Kumar Singh [1], Jeetendra Kumar Nag, Susheela Kushwaha, Shailja Misra-Bhattacharya *

Divisions of Parasitology, Central Drug Research Institute (CSIR), Jankipuram Extension BS10/1, Sector 10, Sitapur Road, Lucknow 226021, UP, India

ARTICLE INFO

Article history:
Received 7 June 2012
Received in revised form 20 February 2013
Accepted 3 May 2013

Keywords:
BmAF-Myo
Bm-TPP
Recombinant protein
Expression

ABSTRACT

Although recombinant vaccines have several advantages over conventional vaccines, protection induced by single antigen vaccines is often inadequate for a multicellular helminth parasite. Therefore, immunoprophylactic efficacy of cocktail antigen vaccines comprised of several combinations of three *Brugia malayi* recombinant proteins BmAF-Myo, Bm-iPGM and Bm-TPP were evaluated. Myosin + TPP and iPGM + TPP provided the best protection upon *B. malayi* infective larval challenge with ~70% reduction in adult worm establishment over non-vaccinated animals that was significantly higher than the protection achieved by any single antigen vaccine. Myosin + iPGM, in contrast did not provide any enhance protection over the single recombinant protein vaccines. Specific IgG, IgM level, IgG antibody subclasses levels (IgG1, IgG2a, IgG2b, IgG3), lymphocyte proliferation, reactive oxygen species level and cytokines level were also determined to elucidate the characteristics of the protective immune responses. Thus the study undertaken provided more insight into the cocktail vaccination approach to combat LF.

1. Introduction

Lymphatic filariasis (LF) is a mosquito-borne neglected tropical disease caused by *Wuchereria bancrofti*, *Brugia malayi* and *Brugia timori*. More than 1.3 billion people in 81 countries worldwide are threatened by LF. Disease pathogenesis is linked to host inflammation invoked by the death of the parasite, causing hydrocele, lymphoedema, and elephantiasis [1]. The current efforts for controlling LF depend mainly on the use of drugs diethylcarbamazine, albendazole and ivermectin which are principally microfilaricidal without much affecting the adult worms, therefore necessitating repeated administrations. Furthermore, signs of emerging drug resistance are becoming increasingly apparent, especially against albendazole and ivermectin [2–4]. The antiwolbachial targeting with antibiotic is also not suitable for mass administration since macrofilaricidal activity requires continuous weeks' long treatment. Therefore, discovery of a new macrofilaricidal drug or a potent vaccine would be the appropriate complementary approaches.

In LF, each parasite stage in the mammalian host interacts with an immunologically distinct compartments, therefore, immune response elicited by each stage has its own distinctive features. Exposure to microfilariae (mf) induces an inflammatory type 1 response whereas infective

* Corresponding author. Tel.: +91 522 2612411 18x4221/4; fax: +91 522 2623405/3938/9504.
E-mail addresses: shailja_cdri@rediffmail.com, shailjacdri@yahoo.com (S. Misra-Bhattacharya).

[1] Both these authors have equal authorship.

0147-9571/$ – see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.cimid.2013.05.003

larve (L3) and adults induce primarily anti-inflammatory type 2 responses. Aggressive immune responses to filarial nematodes occur in some individuals, resulting in chronic pathology, such as elephantiasis. However, the majority of individuals, although having detectable microfilariae in their bloodstreams, are otherwise apparently asymptomatic and have been described as being immunologically tolerant to the parasite. This is thought to be the result of immunomodulation to achieve a situation conducive to both parasite survival and host health. Such people often have dramatically increased levels of immunoglobulin G4 (IgG4) and interleukin-10 (IL-10), with reduced interferon-γ (IFN-γ) production demonstrating the Th2 response. Endemic normal individuals have been reported to mount a Th1-like antifilarial immune response. These observations had led to the conclusion that both the Th1- and the Th2-type responses contribute to effector mechanisms, thus limiting parasite loads, whereas the concomitant suppression of both these responses has to be achieved in order to allow for high parasite loads associated with a low degree of damage to the host. Therefore, developing preventive strategies based on both Th1 and Th2 arms of the immune system appears reasonable.

Attempts have been made to use single recombinant protein [5–8]. However, even in the most successful individual cases, the protection levels obtained were much inferior to that of live attenuated vaccines. Filarial parasites are antigenically complex organism and the immune responses induced by single antigen immunization may not be sufficient to combat the challenge infection. This suggests that development of a cocktail antigen vaccine may be the way forward. Vaccination studies using more than one antigen showed promising results in parasitic diseases such as malaria, leishmaniasis, schistosomiasis and onchocerciasis [9–12]. Few studies have been also reported in the case of LF where cocktail of *B. malayi* recombinant proteins were used. One of these was reported by Vanam et al. [13] where three *B. malayi* recombinant proteins, viz. Bm-TPX (thioredoxin), Bm-TGA (transglutaminase) and Bm-ALT-2 (abundant larval transcript) were administered in various combinations and BmTPX + BmTGA provided better degree of protection as compared to the single antigen immunization. Similarly, a recent study by Anand et al. [14] showed that BmALT-2 and BmVAH (Vespid Allergen Homologue) when given as a cocktail vaccine can confer significant protection (80%) as compared to either of these single protein. Therefore, in the present study, an attempt has been made to investigate the immunoprophylactic potential of *B. malayi* recombinant proteins in cocktails comprised of *B. malayi* adult female heavy chain myosin (BmAF-Myo), independent phosphoglycerate mutase (Bm-iPGM) and trehalose-6-phosphate phosphatase (Bm-TPP).

B. malayi myosin was selected on the basis of its high reactivity with endemic normal human sera from bancroftian endemic area [15]. It is a body wall muscle protein and recently its presence has been shown in excretory secretary product of adult worms [16]. Besides, myosin has been investigated as a vaccine candidate in a number of nematode parasite [17,18]. Excretory-secretary molecules being secreted on host parasite interface are entirely accessible to the host immune system. Bm-iPGM is an enzyme involved in interconversion of 2-phosphoglycerate and 3-phosphoglycerate in the glycolytic/gluconeogenic pathway. It is not reported from mammals and has a sequence and structure different from the 2,3-bisphosphoglycerate-dependent phosphoglycerate mutase (dPGM) found in mammals. Bm-iPGM is also secreted by adult Brugia worms [19]. Thus, these two immunogenic and secretary proteins of *B. malayi* were included as effective recombinant proteins in a multivalent subunit vaccination approach. In addition, Bm-TPP was also included as a component of protein cocktail. TPP is a crucial enzyme in trehalose metabolism. Trehalose disaccharide is an abundant storage sugar in the filarial nematodes that serves as an energy reserve as well as a stress protectant. TPP is also absent in mammals. The present study aims at investigating the possibility of using a cocktail vaccine against lymphatic filariasis using three recombinant proteins of *B. malayi*, BmAF-Myo, Bm-iPGM and Bm-TPP.

2. Materials and methods

2.1. Parasite–host

The experimental animals used in the current study were *Mastomys coucha*, which were maintained under appropriate housing conditions at Laboratory Animal Facility of our Institute. All the animals and experimental protocols involving animal handling were duly approved by the Institutional Animal Ethics Committee (IAEC). *B. malayi* infective larvae (L3) for challenge experiments were recovered from the laboratory bred vector mosquitoes (*Aedes aegypti*) fed on donor *M. coucha* 9 ± 1 day back [20].

2.2. Overexpression and purification of recombinant BmAF-Myo

cDNA coding for *B. malayi* BmAF-Myo was picked up earlier [15] by immunoscreening of adult female *B. malayi* cDNA expression library with human bancroftian sera and subsequently subcloned in pET28b expression vector followed by transformation into DH5α *Escherichia coli* cells (Qiagen). The recombinant plasmid was further transformed into BL21 (DE3) *E. coli* cells (Qiagen) and grown in 5 ml Luria–Bertani (LB) medium overnight (O/N) in the presence of 50 μg/ml of kanamycin at 37 °C with 220 rpm shaking. This O/N grown culture was inoculated in 500 ml of LB medium. The culture was grown in a shaker incubator with constant stirring till A_{600} reached ~0.6. The culture was induced with 0.5 mM isopropyl-β-D-thiogalactopyranoside (IPTG; Sigma) and cells were re-incubated for another 3 h at 30 °C for overexpression of protein containing His tag at the N-terminal. Induced bacterial cells were harvested and disrupted in buffer A (50 mM NaH_2PO_4, 300 mM NaCl, pH 6.5) containing 10 mM imidazole and 1.0 mM phenylmethylene sulfonate (PMSF) as protease inhibitor. Cells were disrupted by sonication and pelleted at $12{,}000 \times g$ for 30 min. Subsequently, the supernatant containing the histidine-tagged recombinant protein was subjected to affinity purification through nickel nitrilotriacetic acid agarose affinity column (Ni-NTA Column, Qiagen). After washing with buffer-A containing