Vaccines to combat river blindness: expression, selection and formulation of vaccines against infection with *Onchocerca volvulus* in a mouse model

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ABSTRACT

Human onchocerciasis is a neglected tropical disease caused by *Onchocerca volvulus* and an important cause of blindness and chronic disability in the developing world. Although mass drug administration of ivermectin has had a profound effect on control of the disease, additional tools are critically needed including the need for a vaccine against onchocerciasis. The objectives of the present study were to: (i) select antigens with known vaccine pedigrees as components of a vaccine; (ii) produce the selected vaccine antigens under controlled conditions, using two expression systems and in one laboratory and (iii) evaluate their vaccine efficacy using a single immunisation protocol in mice. In addition, we tested the hypothesis that joining protective antigens as a fusion protein or in combination, into a multivalent vaccine, would improve the ability of the vaccine to induce protective immunity. Out of eight vaccine candidates tested in this study, *Ov*-103, *Ov*-RAL-2 and *Ov*-CPI-2M were shown to reproducibly induce protective immunity when administered individually, as fusion proteins or in combination. Although there was no increase in the level of protective immunity induced by combining the antigens into one vaccine, these antigens remain strong candidates for inclusion in a vaccine to control onchocerciasis in humans.

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1. Introduction

Human onchocerciasis is a neglected tropical disease caused by *Onchocerca volvulus* and an important cause of blindness, skin disease and chronic disability in the developing world. Through mass drug administration of ivermectin, onchocerciasis has been recognised as a potential candidate for control of morbidity (blindness and skin pathology) and for global elimination by focusing on interruption of transmission (http://www.emro.who.int/neglected-tropical-diseases/ntd-infocus/ntd-roadmap.html, 2014). In some focus areas of the Americas, Mali, Senegal and Nigeria (Kaduna), there has been encouraging evidence that the elimination of onchocerciasis may be possible with mass drug administration of ivermectin, when high levels of therapeutic and geographic coverage over many years have been achieved (Diawara et al., 2009). However, numerous and formidable technical and logistical obstacles must still be overcome before the ambitious goal of elimination can be attained in Africa. These include: (i) the practical complication of treating people for 14–35 years compounds the difficulty of implementing this plan (Winnen et al., 2002; Boatin and Richards, 2006); (ii) experimental studies indicate that susceptibility to reinfection may increase after treatment, further complicating the disruption of the transmission cycle (Duke and Moore, 1968; Abraham et al., 2002; Njongmeta et al., 2004); (iii) recent reports demonstrate that *O. volvulus* in some communities in Africa may have developed resistance to ivermectin (Huang and Prichard, 1999; Kohler, 2001; Awadzi et al., 2004a,b; Ardelli et al., 2005; Bourguinat et al., 2005, 2007; Eng and Prichard, 2005; Osei-Aweneboana et al., 2007); and finally (iv) use of mass drug administration is already compromised in large areas of central Africa where loiasis is co-endemic. Ivermectin cannot be used for the treatment of individuals with high *Loa loa*
microfilaremia due to the risk of developing severe adverse reactions including an encephalopathy (Gardon et al., 1997). Therefore, additional tools are critically needed and include the need for a vaccine against onchocerciasis to complement the present control measures and thus potentially eliminate this infection from humans.

Protective immunity against O. volvulus larvae has been demonstrated in cattle (Tchakoute et al., 2006), mice (Lange et al., 1993) and immuno-epidemiological studies strongly support the hypothesis that protective immunity against onchocerciasis exists in humans (MacDonald et al., 2002), thereby proving the conceptual underpinnings that a vaccine can be produced against this infection. The O. volvulus vaccine would be indicated as a product to protect vulnerable populations living in endemic areas against infection and disease. Reduction in adult worm burden would potentially reduce the number of microfilariae produced by the adult female worms and thus pathology and potentially also the rates of transmission within these endemic regions.

A mouse model was developed for studying immunity to the larval stages of O. volvulus in which larvae are implanted in mice within diffusion chambers (Lange et al., 1993). Protective immunity was demonstrated in this model following immunisation with irradiated infective L3s (Lange et al., 1993; Abraham et al., 2001, 2004). To develop a vaccine with potential clinical application, the model was selected as a moderate throughput means to test recombinant protein or larval vaccines. Recombinant O. volvulus antigens, selected using a variety of criteria, were shown previously to exhibit varying degrees of promise as possible vaccine candidates. In a previously published study, 15 recombinant O. volvulus antigens out of the 44 screened using the O. volvulus-mouse model were found to be protective (Lustigman et al., 2002). Based on the following selection criteria, seven of these protective antigens were selected for further evaluation in the current study: (i) being nematode- or parasite-specific with or without known function. High sequence homology between parasite and mammalian proteins has the potential risk of inducing autoimmunity; (ii) localisation of the corresponding native proteins in larvae by immunoelectron microscopy in one or more regions that are also recognised by antibodies from humans and/or mice with protective immunity to O. volvulus (Lustigman et al., 2003); (iii) being recognised by antibodies from humans with protective immunity or cattle, chimpanzees, mice immunised with irradiated larvae; (iv) the ability of antibodies targeting the parasite antigen to kill larvae in vitro; (v) having homologues that have been shown to also induce protection in other filarial or nematode host–parasite systems (Table 1). In addition, CPI-2 was altered by site-directed mutagenesis to disrupt the asparaginyl endopeptidase inhibitory activity to produced Ov-CPI-2M (Gregory and Maizels, 2008). It has been demonstrated in the Litomosoides sigmodontis/mouse system that this alteration of the antigen enhanced the antigen-specific immune response (Babayan et al., 2012).

The objective of the present study was to produce all candidate vaccine antigens under controlled conditions, using two protein expression systems, Escherichia coli and the yeast Pichia pastoris, and to evaluate their vaccine efficacy using a single harmonised immunisation protocol. In addition, we tested the hypothesis that adding protective vaccine candidates together into a multivalent vaccine would improve the ability of the vaccine to induce protective immunity.

2. Materials and methods

2.1. Expression and purification of O. volvulus vaccine antigens

Yeast codon optimised DNAs encoding for selected O. volvulus vaccine candidates: Ov-B20-C (77 amino acids in the C-terminal region of B20 that were shown to be protective), Ov-RBP-1, Ov-CPI-2, Ov-CPI-2M, Ov-103, Ov-ALT-1, Ov-RAL-2 and Ov-ASP-1, minus the signal peptides at the N-terminus, were synthesized by GenScript (Piscataway, NJ, USA) and subsequently subcloned in-frame into the yeast expression vector pPiNKz-HC (Life Technologies, Carlsbad, CA, USA) with Xhol/KpnI sites and E. coli expression vector pET41a (EMDMillipore, Billerica, MA, USA) with the fusion GST deleted (Ndels/Xhol). The correct open reading frame (ORF) was confirmed by double-stranded sequencing using the vector flanking primers (5′A0X1/CYC1 for pPiNKz-HC and TC7 promoter/ T7 terminator for pET41a). For expression in yeast, the recombinant plasmids were linearized with AflII digestion and then transformed by electroporation into PichiaPink strain I4 with protease A and B knockouts (pep4/pbr11) to prevent P. pastoris-derived protease degradation. Yeast transformants were selected on P. pastoris adenine dropout (PAD) selection plates. The expression of recombinant filarial antigens with hexahistidine (6His)-tag at the C-terminus was induced with 0.5% methanol and the soluble recombinant proteins secreted into the culture were purified with immobilised metal ion affinity chromatography (IMAC) as described previously (Goud et al., 2004). For expression in E. coli, the recombinant constructs cloned into pET41a were transformed into BL21(DE3) (EMDMillipore) and recombinant proteins were induced with 1 mM isopropyl-β-thiogalactoside (IPTG) and purified with IMAC as previously described (Zhan et al., 2002). In order to test the synergistic protection of two or three O. volvulus protective antigen combinations, the selected three protective O. volvulus antigens, Ov-103, Ov-RAL-2 and Ov-CPI-2M, were fused together as a triple antigen (Ov-103-RAL-2-CPI2-M) or as two double antigens (Ov-103-RAL-2 and Ov-RAL-2-CPI2-M) by using a flexible linker (KGDPVPETNQQCPSNTGMTD) obtained from Nε-ASP-1 structure between two pathogenesis-related (RP) domains (Asjojo et al., 2005). The yeast codon optimised fusion DNAs were subcloned into either yeast expression vector pPICZαA (Life Technologies) or E. coli expression vector pET41a (EMD Millipore) with GST knockout. The recombinant fusion proteins were expressed and purified using the same methods described above except for the use of yeast strain P. pastoris X-33 (Zhan et al., 2002). The purity and the molecular weight of purified recombinant proteins were analysed by SDS–PAGE using pre-cast 4–20% Tris-glycine gels (Life Technologies) and stained with Coomassie brilliant blue R-250 (Fisher Scientific, Pittsburg, PA USA).

2.2. Source of parasites and mice

Black flies (Simulium damnosum) were fed on consenting donors that were infected with O. volvulus (Institutional Review Board (IRB) protocol 320 approved by the New York Blood Center, USA and the Kumba, Cameroon IRB). After 7 days the flies were dissected to collect developed L3s, cleaned and cryopreserved in dimethyl sulfoxide and sucrose by using Biocool II computerised freezing equipment (FTS Systems Inc., Stone Ridge, NY, USA) as previously described (Trpis et al., 1993).

Male BALB/cByJ mice were purchased from The Jackson Laboratory (Bar harbour Maine, USA) at 6–8 weeks of age. Mice were kept in the Laboratory Animal Sciences Facility at Thomas Jefferson University, USA. All mice were housed in micro-isolator boxes in a room that was pathogen-free and under temperature, humidity and light cycle controlled conditions. Mice were fed autoclavable rodent chow and given water ad libitum. All protocols using mice were approved by the Institutional Animal Care and Use Committee of Thomas Jefferson University.

2.3. Immunisation and challenge protocol

Mice were immunised with 25 μg of the produced vaccine antigens in 0.1 ml of Tris Buffered Saline (TBS) formulated with 0.1 ml
<table>
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<th>Antigen accession #/kDa</th>
<th>Identity (Function)</th>
<th>Localisation</th>
<th>Immunogenicity</th>
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<td>-Bm-ASP-1 (Bm) Anand et al. (2011)</td>
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<td>-Ac-ASP-2 (Ac) Goud et al. (2004)</td>
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<td>Granules of glandular esophagus; cuticle; channels Joseph et al. (1998)</td>
<td>Human MacDonald et al. (2002)</td>
<td>Jrd – killed Bm L3 and Mf Anand et al. (2011)</td>
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<td>Cuticle; hypodermis; ES product</td>
<td>Cattle anti-Of Abdel-Wahab et al. (1996)</td>
<td>ND</td>
<td>-Cross protection (Av) Jenkins et al. (1996) and Taylor et al. (1995a,b)</td>
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<td>Ov-RBP-1 L277686 (20/ 22)</td>
<td>Novel, nematode specific; Retinoid binding protein</td>
<td>Body wall; ES product Tree et al. (1995)</td>
<td>Human Mpagi et al. (2000)</td>
<td>ND</td>
<td>-Cross protection (Av) (Jenkins et al. (1996) and Taylor et al. (1995a,b)</td>
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Ac, Ancylostoma ceylanicum; As, Ascaris suum; Av, Acanthocheilonema vitreae; Bm, Brugia malayi; ES, excretory–secretory product; Ls, Litomosoides sigmodontis; Mf, microfilaria; ND, Not determined; Ov, Onchocerca volvulus; Ol, O Onchocerca lienalis; Wb, Wuchereria bancrofti.
of 1:5 Rehydragel LV (alum) in PBS (General Chemical, Parsippany, NJ, USA). Mice were immunised s.c. in the nape of the neck, followed by two booster injections 14 and 28 days later.

Cryopreserved L3s were defrosted slowly in two steps, first 15 min on dry ice followed by a 37 °C water bath. Once thawed, the L3s were washed five times in a 1:1 mixture of NCTC-135 and Iscove’s modified Dulbecco’s medium with 100 U of penicillin, 100 μg of streptomycin, 100 μg of gentamicin and 30 μg of chloramphenicol per ml. Diffusion chambers were constructed from 14 mm Lucite rings covered with 5.0 μM pore-size Durapore membranes (EMD Millipore) and fused together using an adhesive containing a 1:1 mixture of 1,2-dichloroethane (Fisher Scientific) and acryloid resin (Rohm and Haas, Philadelphia, PA, USA). The constructed diffusion chambers were then sterilized via 100% ethylene oxide followed by 12 h aeration.

Challenge infections occurred 14 days after the final booster with 25 L3s delivered within a diffusion chamber. The diffusion chambers were implanted in a s.c. pocket on a rear flank of each mouse. Recovery of the chambers was performed 21 days later and larval survival was determined based on mobility and morphology of the remaining larvae. Protective immunity was calculated in two ways: (i) Percentage of reduction in larvae – (average worm survival in control mice – average worm survival in immunised mice)/average worm survival in control mice) × 100. (ii) Host protection was calculated as follows: (number of immunised mice with parasites – number of control mice – total number of immunised mice) × 100. Host cells within the diffusion chamber were collected and analysed by centrifugation onto slides using a Cytospin 3 (Shandon Inc, Pittsburgh, PA, USA). The constructed diffusion chambers were then sterilized via 100% ethylene oxide followed by 12 h aeration.

2.4. ELISA

Serum was collected at the time of recovery for antigen-specific IgG analysis. Maxisorp 96-well plates (Nunc Nalgene International, Rochester, NY, USA) were coated with 2 μg/ml of the immunising recombinant antigen in 50 mM Tris–Cl coating buffer, pH 8.8, overnight at 4 °C. Plates were washed with deionized water between each step. Plates were blocked with borate buffer solution (BBS) (0.17 M boric acid, 0.12 M NaCl, 0.5% tween 20, 0.025% BSA, 1 mM EDTA, pH 8.2) at room temperature for 30 min. Individual sera were diluted to an appropriate starting concentration with BBS and serially diluted; plates were sealed and incubated at 4 °C overnight. Biotinylated IgG (eBioscience, San Diego, CA, USA) was diluted 1:250 in BBS and incubated for 1 h at room temperature, followed by ExtrAvidin Pk (Sigma, St. Louis, MO, USA) which was diluted 1:1000 in BBS and incubated for 30 min at room temperature. One component ABTS peroxidase substrate (KPL, Gaithersburg, MD, USA) was added and O.D.s were read after 30 min at 405 nm in a Bio-Rad iMark Microplate reader (Bio-Rad, Hercules, CA, USA). ELISA data are presented as endpoint titers with the ratio of protein and alum was 1:12.8.

2.5. Statistical analysis

All experiments consisted of five to six mice per group and experiments were performed at least twice with consistent results between experiments. Data were analysed by multifactorial analysis of variance ANOVA with post-hoc Fisher’s Least Significant Difference (LSD) testing in Systat v.11 (Systat Inc., Evanstown, IL, USA). P < 0.05 was considered statistically significant.

3. Results

3.1. Evaluation of antigens expressed in E. coli or P. pastoris for their ability to induce protective immunity

Efforts were made to express the seven selected O. volvulus antigens and Ov-CPI-2 M by both E. coli and P. pastoris. With the exception of OvB20-C, which could only be expressed by E. coli, all of the antigens were expressed by both E. coli and P. pastoris. Of the seven antigens tested, only Ov-103, Ov-RAL-2 and Ov-CPI-2 M induced statistically significant levels of protective immunity in repeated experiments.

3.2. Expression of Ov-103, Ov-RAL-2 and Ov-CPI-2M or fusion recombinant proteins in E. coli and P. pastoris

Onchocerca volvulus vaccine candidates were expressed as soluble recombinant proteins in high yield in E. coli and P. pastoris. E. coli BL21(DE3) after being induced with 0.5% methanol for P. pastoris and 1 mM IPTG for E. coli, and purified with IMAC. Purified recombinant Ov-103, Ov-RAL-2 and Ov-CPI-2M expressed in P. pastoris or E. coli migrated at the same molecular mass as calculated by the coding sequence (14.5 kDa, 17.9 kDa and 16.0 kDa, respectively) on SDS–PAGE and Coomassie staining (Fig. 1).

The fusion recombinant proteins of two or three antigen combination (Ov-103-RAL2, Ov-RAL2-CPI2M and Ov-103-RAL2-CPI2M) were also expressed in P. pastoris and E. coli expression systems as soluble proteins and the purified recombinant fusions were shown at the correct molecular weight as estimated by sequences on SDS–PAGE (50.6 kDa, 32.5 and 35.2 kDa, respectively) (Fig. 2).

Some product-derived degradation was observed in the fusion proteins and these degraded bands could be recognised by anti-His antibody (data not shown). All recombinant proteins including fusions were able to bind completely to alum when the ratio of protein and alum was 1:12.8.

3.3. Immunisation with single antigens to induce protective immunity against O. volvulus

3.3.1. Ov-103

BALB/cByJ mice were immunised with Ov-103 with alum prepared in both P. pastoris and E. coli expression systems. Escherichia coli expression system was used to create the fusion proteins Ov-103-RAL2-CPI2M. A total of 1 μg was loaded for each recombinant protein.
coli expressed protein induced an 8% reduction in larval survival and a 50% level of host protection, whereas mice immunised with the P. pastoris expressed protein had a statistically significant 30% reduction in parasite survival and a 63% level of host protection (Fig. 3A). Differential cell counts were performed at the conclusion of the experiments on the diffusion chamber contents. Comparable numbers of total cells (1.4 × 10^6 ± 1.3 × 10^6), and percentages of lymphocytes (5 ± 7%), neutrophils (52 ± 20%), macrophages (37 ± 15%) and eosinophils (12 ± 14%) were seen in the control and immunised mice. Parasite-specific antibody titers show equivalent endpoint titers for mice immunised with P. pastoris and E. coli expressed Ov-103 when measured against both the P. pastoris and E. coli expressed proteins (Table 2). Correlation analyses were performed between parasite survival and antibody endpoints titers and there were no significant relationships between the amount of antibody produced and the survival of the larvae.

3.3.2. Ov-RAL-2

Mice immunised with E. coli expressed Ov-RAL-2 induced a statistically significant 39% reduction in larval survival and a 64% level of host protection, whereas mice immunised with the P. pastoris expressed protein had a statistically significant 30% reduction in parasite survival and a 63% level of host protection (Fig. 3A). Differential cell counts were performed at the conclusion of the experiments on the diffusion chamber contents. Comparable numbers of total cells (1.4 × 10^6 ± 1.3 × 10^6), and percentages of lymphocytes (5 ± 7%), neutrophils (52 ± 20%), macrophages (37 ± 15%) and eosinophils (12 ± 14%) were seen in the control and immunised mice. Parasite-specific antibody titers show equivalent endpoint titers for mice immunised with P. pastoris and E. coli expressed Ov-103 when measured against both the P. pastoris and E. coli expressed proteins (Table 2). Correlation analyses were performed between parasite survival and antibody endpoints titers and there were no significant relationships between the amount of antibody produced and the survival of the larvae.

3.3.3. Ov-CPI-2M

Immunisation of mice with Ov-CPI-2M expressed in both E. coli and P. pastoris induced statistically significant reductions of 30% in larval survival and 17% levels of host protection (Fig. 3C). As with the other two antigens, differential cell counts showed comparable numbers of total and specific cells in the control and immunised mice, and parasite-specific antibody titers had equivalent endpoints (Table 2). There were no significant correlations between antibody endpoint titers and parasite survival.

3.4. Fusion proteins or concurrent immunisation with Ov-103, Ov-RAL-2 and Ov-CPI-2M

Mice were immunised with Ov-RAL-2/103 fusion protein expressed in P. pastoris and E. coli. Immunisation with E. coli expressed protein significantly reduced larval survival by 21% and provided a 58% level of host protection, whereas immunisation
with *P. pastoris* expressed protein only reduced larval survival by 11% and provided a 45% level of host protection (Fig. 4A). Immunisation with Ov-RAL-2/CPI-2M *E. coli* fusion protein induced protective immunity with parasite reduction at 34% and a 50% level of host protection (Fig. 4B). Analysis of the cells within the diffusion chamber contents showed similar numbers of total cells, lymphocytes, neutrophils, macrophages and eosinophils. Parasite-specific antibody titer endpoints were measured against the individual antigens and the fusion protein. Antibody endpoint titers for the two fusion proteins were significantly higher than the antibody responses in these mice to the individual antigens of which the fusion was composed. The antibody response to Ov-RAL-2 and Ov-CPI-2M by mice immunised with these antigens as part of a fusion were equivalent to the responses seen in mice immunised with antigen individually. However, the parasite-specific antibody titer endpoint to Ov-103 was approximately eight-fold higher in mice immunised with the antigen as part of a fusion compared with immunisation with the individual antigen (Table 2). Once again, there were no significant correlations between antibody endpoints and parasite survival.

A fusion protein consisting of Ov-103, Ov-RAL-2 and Ov-CPI-2M was created to determine whether enhanced protective immunity would be achieved with this triple fused antigen. The Ov-RAL-2/103/CPI-2M fusion was tested in comparison with concurrent immunisation consisting of the three antigens injected simultaneously but at different locations on the mice. Immunisation with the three-antigen fusion protein and the concurrent immunisation resulted in significant levels of protective immunity, with the fusion inducing a 20% reduction in larval survival and a 45% level of host protection and the concurrent immunisation resulting in a 25% reduction in parasite survival and a 64% level of host protection (Fig. 5). Analysis of the cells within the diffusion chamber contents showed similar numbers of total cells, lymphocytes neutrophils, macrophages and eosinophils. Antibody titer endpoints were measured against the individual antigens and the fusion protein. Mice immunised with the three antigens concurrently had

![Table 2](https://www.journals.elsevier.com/)

**Table 2**

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<th>Immunizing Antigen</th>
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*Ov*, *Onchocerca volvulus*. 

![Fig. 4](https://www.journals.elsevier.com/)

**Fig. 4.** Effect of immunisation with fusion antigens on the development of protective immunity to *Onchocerca volvulus* larvae in mice. (A) Ov-RAL-2/103 fusion protein expressed in *Escherichia coli* and *Pichia pastoris* expressed protein; (B) Ov-RAL-2/CPI-2M expressed in *E. coli*. Each dot represents larval recovery from an individual animal. Data presented are mean ± S.D. Asterisk represents statistical difference in larval recoveries; *P* < 0.05.

![Fig. 5](https://www.journals.elsevier.com/)

**Fig. 5.** Comparative effect of immunisation with concurrent injections of *Onchocerca volvulus* Ov-103 (expressed in Pichia pastoris), Ov-RAL-2 (expressed in *Escherichia coli*) and Ov-CPI-2M (expressed in *E. coli*) compared with immunisation with the combined fusion antigen Ov-RAL-2/103/CPI-2M (expressed in *E. coli*). Each dot represents larval recovery from an individual animal. Data presented are mean ± S.D. Asterisk represents statistical difference in larval recoveries; *P* < 0.05.
antibody endpoint titers to the three antigens that were comparable with those seen in mice immunised with the three individual antigens (Table 2). Mice immunised with the three-antigen fusion protein had endpoint titers to the single antigens that were comparable with the titers seen in mice immunised with individual antigens. Antibody endpoint titers for the three-antigen fusion protein were significantly higher than the antibody responses in these mice to the individual antigens of which the fusion was composed (Table 2). There were no significant correlations between antibody endpoints and parasite survival.

4. Discussion

Previous studies have identified several antigens with either proven or potential efficacy in a vaccine against infection with O. volvulus. One of the challenges in comparing these studies is the range of approaches used to produce the recombinant antigens, which were performed by laboratories across the USA and Europe, and the immunisation protocols used to test these antigens (Lustigman et al., 2002). The objective of the present study was to produce the eight tested vaccine antigens under controlled conditions, using two expression systems and in one laboratory, and to evaluate their vaccine efficacy using a single immunisation protocol. In addition, we tested the hypothesis that joining protective antigens as a fusion protein or in combination into a multivalent vaccine would improve the ability of the vaccine to induce protective immunity.

Out of the eight antigens tested, only Ov-103, Ov-RAL-2 and Ov-CPI-2M were able to repeatedly induce protective immunity under the experimental conditions used in the present study. There are numerous possible explanations for why the other vaccine antigens did not induce protective immunity as was previously reported, including the immunisation regimen, adjuvant formulation, expression system and type of challenge infection. All of the tested antigens evoked significant antibody responses, which suggests that the immunisation regimen was adequate. There may have been inadequate activation of other elements of the immune response that collaborate with antibody to kill the parasites.

Previous studies on the role of adjuvants in development of recombinant-antigen vaccines against O. volvulus have demonstrated that either alum (Abraham et al., 2001) or FCA was required (McCarthy et al., 2002) or that either adjuvant was successful at enhancing the capability of the antigen to induce protective immunity to the parasite (MacDonald et al., 2004). Immunity to O. volvulus induced by irradiated larvae is dependent on Th2 responses (Lange et al., 1994; Abraham et al., 2004) and alum has the recognised ability to stimulate humoral immunity and strong Th2 responses (Kenney et al., 1989; Brewer et al., 1996; Yip et al., 1999). In addition, alum remains one of the few adjuvants that is commonly used in human vaccines, whereas FCA is not recommended for use in humans. Alum was therefore selected for use in this study due to its predilection for inducing Th2 responses and due to its acknowledged safety in humans.

Two different expression systems were used in this study. Escherichia coli was selected as it was the original system in which the antigens were produced. Pichia pastoris was used as it provides an expression system in which there are post-translational modifications such as glycosylation that will potentially yield antigens more closely resembling the native proteins (Hohenblum et al., 2004). Seven of the eight tested O. volvulus antigens were successfully produced in both expression systems with Ov-B20-C only produced in E. coli. The finding that some antigens are preferentially expressed in one system over the other has been observed with other antigens (Zhang et al., 2006). Interestingly, all eight antigens, regardless of the expression system, induced robust antibody responses. The three antigens that induced protective immunity had equivalent antibody endpoint titers regardless of the expression system and there was comparable recognition of the antigens from the two expression systems regardless of the source of the immunising antigen. Yet, mice immunised with P. pastoris expressed Ov-103 had a statistically significant reduction in parasite survival but not with E. coli expressed antigen. Ov-RAL-2 induced a statistically significant reduction in parasite survival only if produced by E. coli and Ov-CPI-2M produced in either Pichia or E. coli induced protective immunity. In other studies, no differences were observed in the immune responses induced by E. coli and Pichia expressed antigens (Giessing et al., 2005), whereas some antigens were more immunogenic if produced in E. coli compared with Pichia (Kastenmuller et al., 2013) and yet other antigens were more antigenic if produced in Pichia compared with E. coli (Yang et al., 2012).

Two metrics were used to measure the development of protective immunity. “Larval survival” assessed the number of larvae that were killed in immunised compared with control mice and describes the level of protective immunity within individual mice. “Host protection” measured the percentage of mice that developed significant resistance to the infection and reflects the level of protective immunity within the host population. Immunisation with the three protective antigens resulted in equivalent levels of protective immunity based on both metrics. Efforts to resolve why an antigen expressed in one system induced protective immunity, whereas expression in the other system did not induce protective immunity, were not informative. There were no correlations between antigen-specific antibody levels and the number of larvae killed in a mouse. The number and types of cells that migrated into the parasite microenvironment did not vary between control and immunised animals nor between Immunised mice that developed protective immunity and immunised mice that did not develop protective immunity. Further analysis of the immune response is required to resolve the molecular mechanism of parasite killing induced by the recombinant antigens. Antibody class and subclass responses, as well as the binding affinity of the antigen-specific antibodies, may govern the potency of the antibodies. Cell analysis needs to be expanded to observe the cells in the parasite microenvironment at the time prior to parasite killing and at the time of larval death. Furthermore, it has been shown that effector cells, eosinophils (Lee et al., 2010), macrophages (Bonne-Annee et al., 2013) and neutrophils (Tsuda et al., 2004; Christoffersson et al., 2012) undergo specific forms of activation dependent on the host or pathogen factors to which those are exposed. It is possible that effector cells are recruited to the parasite microenvironment, but are ineffectual due to their activation status. It is hypothesised that in mice with protective immunity, cells are recruited to the parasite and specifically activated into a state in which they can participate with antibody in the killing process.

Multivalent vaccines have been successfully developed against the filarial worm Brugia malayi (Anand et al., 2011; Joseph and Ramaswamy, 2013; Shrivastava et al., 2013) and immunisation of calves with a multivalent vaccine against Onchocerca ochengi reduced the development of patency as determined by a decrease in the number of animals with microfilariae in the skin (Makepeace et al., 2009). It was hypothesised that immunising mice against O. volvulus with two or three of the protective antigens would induce elevated levels of protective immunity. Concurrent immunisation of mice with all three protective antigens did not induce increased levels of protective immunity compared with immunisation with the individual antigens. In a previous study utilising three recombinant O. volvulus antigens, immunisation with the three antigens as a cocktail also resulted in levels of protective immunity equivalent to that induced by the component individual antigens (Abraham et al., 2001). Analysis of antibody responses following
immunisation with the cocktail of three antigens revealed that some of the antibody responses were reduced compared with that seen in mice immunised with individual antigens (Abraham et al., 2001). It was hypothesised that there was competition between the antigens in the cocktail, with some of the antigens dominating the immune response (Abraham et al., 2001). Therefore, in the current study the three antigens were injected concurrently and in different locations on the mice. The result was antibody responses to the three antigens that were not diminished, yet did not result in elevated levels of protective immunity.

As an alternative approach, fusion proteins consisting of two or three of the protective recombinant antigens were tested for their efficacy in the vaccine against infection with O. volvulus. Mice immunised with Ov-RAL-2/103, Ov-RAL-2/ CPI-2M or Ov-103/RAL-2/CPI-2M fusion proteins had significant levels of larval killing and host protection. Mice immunised with the two or three-antigen fusion proteins had endpoint titers to the single antigens that were at least comparable with the titers seen in mice immunised with individual antigens. Antibody endpoint titers for the fusion proteins were significantly higher than the antibody responses in these mice to the individual antigens of which the fusions were composed. Even in the face of the significantly enhanced antibody titers the levels of protective immunity were not enhanced in the mice immunised with the double or triple fusion proteins. Fusion protein vaccines developed against Plasmodium falciparum have better efficacy than antigen cocktails (Faber et al., 2007, 2013; Alaro et al., 2013). A multivalent fusion protein vaccine against B. malayi conferred 95% protection in mice (Dakshinamoorthy et al., 2013). Interestingly, it was concluded in a recent review that mice immunised against B. malayi have a high degree of natural resistance to infection with B. malayi (Alaro et al., 2013). This high degree of natural resistance of mice to B. malayi infection has been observed in the current study, induced by the three protective antigens, do not reflect the true potential of these vaccines to induce protective immunity in the clinical setting. Although the present data do not take into account repeated exposures which occur in the field during ongoing transmission, we still submit that a vaccine composed of these three antigens will function in humans at levels greatly exceeding those seen in mice and will protect the vaccinated individuals from infection caused by O. volvulus.

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References


