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## **Future prospects and challenges of vaccines against filariasis**

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### **Abstract**

Filarial infections remain a major public health and socio-economic problem across the tropics, despite considerable effort to reduce disease burden or regionally eliminate the infection with mass drug administration programs. The sustainability of these programs is now open to question due to a range of issues, not least of which is emerging evidence for drug resistance. Vaccination, if developed appropriately, remains the most cost-effective means of long term disease control. The rationale for the feasibility of vaccination against filarial parasites including onchocerciasis (river blindness, *Onchocerca volvulus*)

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and lymphatic filariasis (*Wuchereria bancrofti* or *Brugia malayi*) is founded on evidence both from humans and animal models for the development of protective immunity. Nonetheless, enormous challenges need to be faced in terms of overcoming parasite induced suppression without inducing pathology as well as the need to both recognise and tackle evolutionary and ecological obstacles to successful vaccine development. Nonetheless, new technological advances in addition to systems biology approaches offer hope that optimal immune responses can be induced that will prevent infection, disease and/or transmission.

## Introduction

Filariasis presents major public health problems in more than 90 developing countries. Current control of these infections relies on mass treatment with ivermectin (onchocerciasis) or diethylcarbamazine (lymphatic filariasis), either alone or in combination with albendazole. Major international initiatives such as the African Programme for Onchocerciasis Control (APOC), the Onchocerciasis Elimination Program for the Americas (OEPA) and the Global Programme to Eliminate Lymphatic Filariasis (GPELF) have been established to provide sustained delivery of treatment with the aim of eliminating these infections as public health problems (1, 2). However, despite the demonstrable achievements of these programmes (1, 3), an estimated 160 million people remain infected and a further billion are at risk of infection (4). This situation argues strongly for an alternate control strategy.

Vaccination, where it exists, is the most cost effective method of controlling an infection. Although eight filarial species that can parasitise humans, onchocerciasis (*O. volvulus*) and lymphatic filariasis (*W. bancrofti* and *B. malayi*) are the targets of vaccine research efforts, as they are the major causes of morbidity. *Proof-of-Principle* of vaccination against filariae has been demonstrated in animal models with live attenuated (5, 6) and with recombinant and DNA vaccines (7), and there is considerable knowledge about the mechanisms that underlie vaccine-induced protective immunity as discussed below. This knowledge, combined with the application of post-genomic technologies to antigen identification, analysis and delivery, means there has never been greater optimism nor a better opportunity to develop vaccines against filarial infections.

## The need for a vaccine

Existing drugs and control programmes have important limitations (4, 8, 9). The single annual dose of ivermectin that is used in mass onchocerciasis treatment programmes is very effective at killing microfilariae and hence reducing morbidity associated with skin and eye disease. However, ivermectin does not kill adult worms and while prolonged use can reduce female fecundity, sterilisation may not be permanent and once treatment stops microfilariae can repopulate the skin and return to levels measure before treatment within 1 year. This increases the risk of new disease episodes for the individual as well as resumption of transmission in the community. Another limitation of ivermectin is that while generally well tolerated and safe for use, in a large area of equatorial west and central Africa (10), where *O. volvulus* is co-endemic with *Loa loa*, there is a risk of serious adverse reactions to treatment, including coma and death associated with massive death of *L. Loa* microfilariae (11).

Another major concern is emergence of resistance to ivermectin. It is now over 25 years since ivermectin has been in regular and wide-spread use against onchocerciasis and there is a small but growing literature describing genetic selection of *O. volvulus* by this drug (12). The persistence of microfilariae in the skin after repeated treatment with ivermectin (13) raises questions about the emergence of resistance and although some debate surrounds the explanations for the so-called sub-optimal responses to ivermectin, experience in the veterinary field points towards the inevitable development of drug resistance among filariae.

Fortunately there has been a long-standing search for new drugs and, in particular, effective macrofilaricides. In this context, a major advance was made with the demonstration, in cattle, that tetracycline treatment aimed at the filarial endosymbiont rickettsia *Wolbachia pipensis* resulted in death of adult *O. ochengi* (14). *Wolbachia* is found in the majority of filariae species (15), but crucially it is not present in *L. loa* (16). In humans, a 6 week course of doxycycline resulted in death of 60% adult *O. volvulus* (17). Doxycycline is safe, well tolerated and its absence from *L. loa* provides an alternative for control of onchocerciasis in areas co-endemic for loiasis (18).

For treatment of lymphatic filariasis, diethylcarbamazine (DEC) or ivermectin are used, either alone or in combination with albendazole (19). A single dose of DEC significantly reduces blood microfilaraemia over a year, but not all adult worms are killed (20). Adverse

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reactions may occur and the severity of local inflammatory responses associated with *O. volvulus* microfilariae in the eye preclude use of DEC in regions of Africa where lymphatic filariasis and onchocerciasis are co-endemic. To avoid such risks, ivermectin is given alone or in combination with albendazole. However, *W. bancrofti* and *L. loa* are co-endemic in 10 African countries and, as mentioned in the case of onchocerciasis, the risk of severe adverse reactions associated with death of *L. loa* microfilariae following ivermectin use has prevented the initiation of lymphatic filariasis mass treatment programmes (21).

Doxycycline again provides an alternate therapy against *W. bancrofti* and *B. malayi* (9, 22) with the additional benefit of its ability to ameliorate pathology (23, 24). However, doxycycline can not be given to children under the age of 9 (17) who, together with pregnant women and lactating mothers, are excluded from mass treatment programmes. Children below 5 years are also excluded from ivermectin treatment. This leaves a significant proportion of individuals exposed to infection. For example, in Gabon where the entire country is co-endemic for *O. volvulus* and *L. loa* (10), 20% of the population is under 10 years (United Nations, <http://esa.un.org/unpd/wpp/index.htm>), and similar age profiles are found throughout filarial endemic regions of Africa. Therefore, untreated children represent a large reservoir of microfilariae that can contribute to transmission. Furthermore, for the individual, the consequences of not receiving treatment would be the prospect of developing progressive filarial disease and more general long-term health problems as well as associated socio-economic disadvantage.

The proposition that mass drug treatment can eliminate filarial infections from Africa is contentious (25, 26). For mass treatment to be effective at reducing transmission of lymphatic filariasis it must be maintained for at least 5 years, while it may take at 15-17 years to reduce transmission of onchocerciasis (WHO estimates). However, reduced transmission does not mean control or elimination of these diseases as public health problems.

Introduction of doxycycline as a prescribed treatment for both diseases would make a difference for the individual and community but elimination of these infections from Africa using these tools only seems most unlikely. Vaccination would make a major contribution to control of filarial infections and its inclusion in national immunisation programmes would provide pre-school children with protection.

## Evidence for Protective Immunity

### *In humans*

Filarial infections are characterised by a spectrum of clinical and parasitological presentations (4). At one end of this spectrum are the severe skin lesions of onchocerciasis and the elephantiasis of lymphatic filariasis. Patients presenting with these pathologies have strong Th2 type responses including elevated levels of IgE with absent or low microfilaraemia. Inflammatory responses underlie the pathology and physiological changes seen in hyper-reactive (localised) onchocerciasis (sowda) (27) and lymphoedema and elephantiasis of lymphatic filariasis (28). Fortunately, such severe disease does not afflict a majority of infected individuals and many present with milder symptoms, despite the presence of sometimes massive numbers of microfilariae in the skin (onchocerciasis) or blood (lymphatic filariasis). Such individuals frequently exhibit a marked filarial-specific T-cell unresponsiveness with impairment of both Th1 and Th2 pathways (29). Initially, this regulation was ascribed to predominant IL10 responses but it is becoming clear that a range of regulatory events are involved including TGF- $\beta$ , CTLA-4, natural regulatory T cells and alternatively activated myeloid cells, with enhanced production of IgG4 characteristic of the downmodulated immune response (29, 30).

Somewhere [immunologically] between the hyper-reactive diseased state and immunosuppressed parasitised individuals lie a third but rare group of individuals who despite having lived their entire lives (or prolonged periods) in areas endemic for onchocerciasis or lymphatic filariasis, and continually exposed to infected vectors, show no parasitological or clinical signs of infection. These individuals are described as *putative immune* or *endemic normals* (31) and are frequently cited as providing evidence for the existence of protective immunity against filarial infections. If true, these individuals should provide information about effector mechanisms capable of destroying invading L3 that could be vital for successful development of vaccines.

In one study, endemic normals were found to produce stronger Th2 responses (e. g. more IL5) against L3 antigens than individuals with patent *O. volvulus* infections (32). However, half of the endemic normals tested also had demonstrable Th1 responses as judged by production of IFN $\gamma$ . Such mixed Th1/Th2 responses had been previously observed in endemic normals in other cross-sectional studies of onchocerciasis and lymphatic filariasis (e. g. (33-35)), and can be seen in murine models of protection (see below). Where

investigated, endemic normals, lack the elevated levels of filarial specific IgG4 associated with a regulated Th2 environment seen in individuals with patent infections and presenting with no or mild disease. In general, endemic normals have low IgG4/IgE ratios and in one study (36, 37) this profile was associated with elevated  $\gamma$ -interferon levels and again indicative of a mixed Th1/Th2 response. The concept, or reality, of endemic normals remains controversial because there is no absolute surety that an individual is not infected, nor is it practical and ethical to carry out the longitudinal studies that may resolve unanswered questions. It is this situation that benefits from and necessitates animal experimentation.

### **Cattle model**

Putative immune humans and endemic normals have direct counterparts in cattle exposed to *O. ochengi* (38, 39). *O. ochengi* is the closest known relative to *O. volvulus* (40) and transmitted by the same zoophilic blackfly vector (*Simulium damnosum sl.*). The parasite has a wide distribution in sub-saharan Africa and an epidemiological study in Cameroon (41) has even suggested that the reduced prevalence of onchocerciasis was a consequence of exposure to *O. ochengi* L3: an example of zoonophylaxis (42)?

Adult *O. ochengi* are found in intra-dermal nodules that can be easily quantified as a measure of drug or vaccine efficacy (43)). The immunological cross-protection suggested by Wahl et al. was subsequently demonstrated following vaccination of cattle with live *O. volvulus* L3 (44). Furthermore, vaccination with irradiated *O. ochengi* L3 induced good protection against natural challenge (64% reduction in mean nodule load compared with controls) and 100% protection against experimental challenge (45).

The cellular basis of this protection has yet to be established. Early parasite-specific response of cattle infected with *O. ochengi* exhibit a mixed Th1/Th2 profile but the onset of patency is coincident with marked inhibition of IFN $\gamma$  and IL4 production which has similarities to the hypo-responsiveness seen in patent human filarial infections (46). Eosinophils have been implicated in the killing of adult *O. ochengi* following temporary depletion of *Wolbachia* by tetracycline (47), but whether such a Th2-driven response follows vaccination of cattle with irradiated L3 remains to be demonstrated.

### **Rodent models**

Vaccination of cattle with irradiation-attenuated L3 follows an established route to immunisation against parasites that was first exploited in the late 1950s when irradiated L3 larvae of *Dictyocaulus viviparus* were introduced as a commercial vaccine against cattle lung worm (48). Subsequently, immunisation with irradiation-attenuated infective larvae has been shown to stimulate significant levels of protection against both plathyhelminth (e. g. schistosomes (49)) and nematode species, including filariae (50), and despite the incomplete nature of this protection, irradiated larvae remain the gold standard by which other vaccination protocols are measured. Just how irradiated L3 larvae drive protective responses remains unclear. Immunisation with soluble filarial antigens induce good antibody responses but these are not protective and infection with non-irradiated L3 larvae rarely evoke a demonstrable protective response. Early studies performed with irradiated L3 of *O. volvulus* (51) and *B. malayi* (52) in mice suggested that protection is associated with a Th2 response that may involve IgE and eosinophils (53, 54): a conclusion also drawn from studies on *Acanthocheilonema viteae* in *Meriones unguiculatus* (55) and *B. pahangi* in cats (56).

The absence of reagents has limited more detailed immunological investigation of filarial infections in cats and jirds and consequently most attention has focused on murine models. However, the L3 of human filariae can not mature and produce patent infections in immunocompetent mice and this has hampered immunological investigations. This constraint was removed when Bain and colleagues adapted *L. sigmodontis*, a natural filariae of the cotton rat *Sigmodon hispidus*, to the laboratory mouse (57) and opened filarial research to the full extent of immunological tools that are available for laboratory mice, including genetic manipulation of the host. Patent infections can develop in BALB/c mice while C57BL/6 mice are resistant to patent infection (58). Amicrofilaraemic infection also occur in BALB/c mice and thus the parasitological presentations seen in human filarial infections are replicated by *L. sigmodontis* in mice.

The *L. sigmodontis* model has shown that resistance can be abrogated by knocking out the IL-4 gene (59). Indeed, a consistent finding from both *L. sigmodontis* studies and earlier work using *B. malayi* in mice is that IL4 and/or IL13 are required for containment of the larval stages, both incoming L3s and microfilariae (60-63). Such results are in keeping with the general finding that Th2 responses are needed for immunity to most helminth species (64).

However, significant complexity is added by the fact that BALB/c mice, which are genetically susceptible, produce IL-4 in abundance (59). Progress towards resolution of this conundrum was made by the demonstration that susceptibility relies on the establishment of an early CD4+FoxP3+ T regulatory response (65) and that long term adult parasite survival requires both T regulatory cells and maintenance of a T-cell hypo-responsive state (66). These observations are arguably some of the most important in filarial immunology because recognition of involvement of Tregs and associated regulatory pathways provide an explanation for the spectrum of parasitological and clinical presentations seen in human filarial infections. Furthermore, abrogation of all or part of these regulatory processes may provide the key to successful vaccination.

Mice vaccinated with irradiated *L. sigmodontis* L3 routinely lead to 70% reduction in worm burden when compared with controls when the challenge infection is performed two weeks after the immunisation (5, 67), although when the challenge is given five months after vaccination, protection falls to 50–55% (6). This protection involves an antibody-dependent eosinophil-mediated process in the skin (68–71), and occurs immediately after the delivery of the infective L3 (5). Involvement of antibody and eosinophils in protective immunity is consistent with previous studies using *O. volvulus* larvae in mice (53, 54) and *A. viteae* in jirds (72).

To date, most studies have focussed on the incoming L3s but there are good arguments for targeting microfilariae, both to block transmission and to reduce risk of pathology (e. g. onchocerciasis skin disease or tropical pulmonary eosinophilia associated with lymphatic filariasis). Studies in which microfilaria are directly injected in the blood stream of resistant mice has shown that early and rapid (6 day) clearance of microfilariae is an innate response that does not depend on production of nitric oxide (63, 73, 74). A fascinating twist to the story is that the presence of adult females enhances microfilarial survival in the blood which is associated with elevated levels of IL10 (63). The timing of these events is indicative of T-independent responses but there is also good evidence that IL4 or IL13 driven pathways are necessary for the control of microfilaraemia following natural challenge with *L. sigmodontis* L3 in both susceptible and resistant strains (59, 61, 75, 76). Some argue that the IL4 responses negatively impact on fecundity rather than being responsible for direct attack on microfilariae (59, 61, 77). However, IL4 may also act through promoting antibody dependent cellular cytotoxicity responses that can mediate clearance of microfilariae (78).

Critically, different effector mechanism may be operating in different models and, at the level of the individual, there may be temporal changes in the relative dominance of one effector response over another during the course of an infection. Nonetheless, there is clear evidence that the parasite-driven immunoregulation plays a central role in the parasitological outcome of filarial infections and that both innate and acquired responses are under the influence of the regulatory networks that determine clinical outcomes (64). For the most part, infected hosts manage to maintain a balance of regulatory and effector responses that, while not completely controlling infection, can prevent severe disease. Sometimes, for a fortunate few, this balance favours complete elimination of infection.

## **The Challenges**

### ***Vaccine Targets***

Given the complexity of immune responses that are needed to resist chronic infection and target different parasite life stages without the induction of immunopathology, it is no surprise that there is still no vaccine against filariasis. Efforts to develop vaccines have focussed on a range of proteins that are associated with immune protection in animal models or are abundant at the L3 stage. These experimental vaccines produced either as recombinant proteins or DNA plasmids give highly variable protection but can sometimes give impressive results especially when combined into multivalent vaccines (7, 79, 80). Defining protective antigens is a daunting task when one considers that nematodes have genome sizes nearly as large as their hosts and indeed many more candidates will come from the systems biology approach discussed below. A particular problem for filarial parasites is which stage to target? Most efforts have focussed on the incoming L3s but as discussed above there are good arguments for targeting MF, both to block transmission and to reduce pathology, particularly in the case of onchocerciasis. Immunity induced by irradiated L3, which is the current gold standard of antifilarial vaccination, is often partial and acts primarily during the early stages of infection (5). In genetically susceptible hosts, larvae that survive vaccination migrate away from the skin and thereby escape further vaccine-induced destruction. This is significant in the light of our recent study demonstrating that the nematodes are able to respond to the presence of eosinophils by accelerating their development and increasing their fecundity (6, 81). As a consequence, the parasite's response to host immunity may reduce vaccine efficacy unless vaccines can also target later life stages more efficiently.

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As described above, the biggest challenge to effective vaccination may be parasite-driven immunoregulation. Collective work to date has led to the proposal that specifically targeting parasite-derived immune modulators (82, 83) could allow the host to mount a protective Th2 response. Although such a vaccine may allow the host to generate protective responses more easily against multiple parasite life stages, experimental manipulation shows that the balance between regulatory and effector cells is not only driven by the parasite but, in large part, is determined by the host (66). Thus, simply reducing parasite immune modulation may not be sufficient for a vaccine to induce full protection. Further, overcoming parasite-induced regulation may be an important pre-requisite for effective vaccination, although some level of regulation must be maintained to avoid inducing pathology. Indeed, mammals may have evolved to respond to nematodes with regulatory rather than effector mechanisms because resistance and the associated immunopathology is too costly. So it becomes essential to understand the mechanisms of parasite tolerance (84), such as T regulatory cells, and determine whether vaccines can work with or despite them.

### **Pathology**

The severe pathology seen in onchocerciasis and lymphatic filariasis is associated with elevated IgE responses and yet many nematode antigens and potential vaccine targets are themselves allergens with the capacity to induce IgE. For example, one of the vaccine candidates that has been considered a strong contender for inclusion in any anti-filarial vaccine are the venom allergen homologues (VALs) also called *Ancylostoma* secreted proteins (ASP). Considerable hope was placed on the idea that the filarial field could *piggy back* on the human trials underway using the *Necator americanus* VAL for immunisation against hookworm infection (85). However, these trials faced a set back as individuals with pre-existing IgE responses to the vaccine candidate exhibited allergic responses that led to the discontinuation of those trials (86). The hookworm vaccine research provides both lessons and guidance for the future. Trials with two recombinant antigens that are protective in animal models but do not induce IgE in humans are moving forward (86). This suggests that an approach in which protection is generated by non-IgE responses is feasible and can avoid the risks associated with using allergens as vaccine candidates. Nonetheless, considerably more immunological research is needed to generate a fuller understanding of how to generate a protective response and avoid allergic hypersensitivity.

## **Adjuvants**

A majority of protection studies support the concept that induction of the Th2 arm of the immune system is an appropriate strategy for vaccination against helminths including filariasis. The basic parameters that lead to Th2 cell activation are well known (87) but there are still major gaps in our understanding of what specific components are needed to generate and fine tune the response. For example, despite indications to the contrary, dendritic cells (DC) are needed to initiate a Th2 response (88) but the specific signals provided to the DC, and by the DC to the naive T cell, are still unclear (89). Further, we now understand that Th2 immunity does not reflect a single T cell phenotype but that Th2 cells can be fine tuned to produce a very different array of cytokines and newly defined T cell subsets such as Th9 may be involved in anti-helminth immunity. Determining which type of immune response is the most effective against different filarial stages of parasites is no small challenge.

Further, unlike the more advanced fields of viral and bacterial vaccinology in which the triggers of innate immunity such as TLRs are known, we do not know the innate signals that promote Th2 responses against filariae. Nonetheless, we do know that the cytokine milieu is a critical factor in inducing a particular T cell subset (87). With greater knowledge of how both effector and regulatory pathways influence expression of protective immunity against filariae the potential exists to orchestrate an effective response by delivery of the appropriate cytokines and secondary signals to drive naive T cells towards the Th2 lineage.

From the viewpoint of helminth vaccination, alum, one of the very few adjuvants licensed for use in humans is very effective at driving a Th2 response) although how this is achieved is unknown (90). Fortunately, this is an active research area and the findings may contribute to the development of new more effective Th2-inducing adjuvants.

## **Meeting the challenges**

### ***Molecular engineering***

Our growing understanding of the mechanisms that enable parasites to evade the lethal effects of their host's immune systems and particularly their ability to modulate effector responses provide an opportunity to target specific parasite and host pathways and interactions. DNA technology allows the use of two complementary strategies: immunising against immunomodulators, and refining adjuvants. Parasite excretory-secretory products

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have been identified as a source of immunomodulators but these molecules can be poorly immunogenic or even block their own processing by antigen presenting cells (APC) as may be the case for cystatins (91, 92). Such observations indicated that careful structural analyses may be required to identify regions (e. g. enzymatic or inhibitory sites, post-translational modifications) responsible for immunomodulatory activity so that these may be removed and the immunogenicity of protein enhanced. For example, *B. malayi* cystatins have two protease-inhibitory sites which can be inactivated by point mutations (92). In the *L. sigmodontis* model, the inactivated form of the recombinant protein is proving to be far more immunogenic, and thus more protective, than the native protein (unpublished data).

To further overcome the poor immunogenicity of parasite products, vaccines may be formulated or constructed to specifically target antigen presenting cells such as DC (93) and thereby induce the appropriate level of response through manipulation of co-stimulatory pathways. Such approaches are, in principle, applicable to a wide range of diseases, and a wide range on immune pathways, whether they utilise recombinant protein or DNA vaccines.

### **Systems biology**

The widespread availability of gene sequencing combined with the functional analysis of specific parasite proteins such as those the parasite secretes into its environment, is allowing the identification of many new vaccine targets. This approach is being expanded exponentially by the ever greater affordability of high throughput sequencing platforms which is currently being applied to filarial and host transcriptomes (<http://www.filaria.eu/projects/projects/epiaf.html>). We are reaching a point where the greatest difficulty is choosing which candidate genes to prioritise. Further, the exponential increase in our understanding of immune gene function and regulation, of cellular activation cascades and of determinants of resistance and pathology offers a myriad of possible vaccine approaches. A number of parasite immune modulatory molecules are currently being tested. However, only a fraction of the total molecular repertoire can be expected to be tested experimentally and the complexity of host effector and regulatory systems similarly limits direct manipulation. The solution to this problem may lie with application of systems and pathway analysis (94). This approach, which is already providing exceptional benefit to investigators of viral (e. g. (95)) and bacterial diseases (e. g. (96, 97)), can now be exploited to help disentangle the interactions occurring between the immune system and eukaryotic parasites.

Immunologists, and in particular those who wish to manipulate immune responses to combat disease, are increasingly requiring powerful analytical methods to decide which elements of the immune system to target, whether individual genes or entire pathways. Filarial nematodes trigger type-2 immune responses, which are necessary for controlling the infection. However, there is a trade-off between parasite killing and the risk of type-2 immunopathology, and most pathology caused by filarial parasites stems from poorly regulated immune responses. Designing vaccines against these pathogens therefore would greatly benefit from a *systems biology* approach to the determinants of disease and/or resistance. Such an approach could therefore inform of the risks of reducing host-generated immune regulation (e. g. by targeting the TGF- $\beta$  pathway) or of the immune pathways that may still mediate protection but without triggering parasite developmental cues Babayan et al., 2010, #60075}. To generate and test hypotheses about what constitutes a safe and protective immune response will require strong interdisciplinary links. Indeed, collaboration between classical parasitologists, medics, systems biologists, and molecular biologists is no longer a luxury but a necessity.

### ***Biological Constraints - the long view***

The potential for a revolution in vaccine design is huge and the use of recombinant protein or DNA vaccines along with our technological advances in basic vaccine biology will solve many of the logistical problems that prevented the use of attenuated vaccines against filariasis.

### ***Evolution***

However, it should be remembered that parasites have evolved sophisticated mechanisms to evade their host's immune responses. There is a risk that imperfect vaccination may drive the evolution of filarial adaptations that allow their continued transmission. For instance, a vaccine that targets one immunosuppressive protein may select parasites that favour a different immunosuppressive molecule. Further, by reducing parasite longevity we could select parasites with shorter natural lifespans and higher fecundity (98), thereby potentially increasing transmission and/or pathology. Our work on *L. sigmodontis* immune-dependant phenotypic plasticity has shown that such life history shifts can occur within the parasite's lifespan (81). Although combined drug and vaccine treatment may reduce vaccine-escape evolution, such optimism may be unwarranted as persistent drug pressure can lead to the evolution of resistance at little or no fitness cost to parasites (99). Fortunately, evolutionary

theory can inform both vaccine design and intervention strategies, for instance by providing predictive power to a given vaccination approach, and help weigh trade-offs between mitigation of pathology and elimination of parasite transmission (84, 100, 101).

### **Ecology**

Finally, there is the further challenge of leaving the laboratory for the *real world*. Vaccines are required to protect a sufficient number of individuals whose genetic susceptibility to infection varies widely (102, 103). In addition to variation in host immune genotype, protective immunity can vary as a result of its prior interactions with other pathogens as well as nutritional status, age and gender (104). Such sources of variation are known to affect host immune responses and susceptibility to infection (105, 106), and thus may degrade the efficacy of anti-filarial vaccines below their performance in the laboratory. This requires greater investment in human and non laboratory-based animal immunological studies and particularly longitudinal investigations at the community level, as well as acquisition of knowledge of the genetic make-up of parasite populations in relation to parasite survival and transmission.

### **Conclusion**

Despite these obstacles, there is room for optimism. The existence of natural immunity in people gives hope that vaccines can be developed as does the success of vaccines in animal models. Knowledge of anti-filarial immunity has made enormous advances in recent years and development of the rodent filarial nematode *L. sigmodontis* has allowed the full power of mouse genetics and immunology to be applied to anti-filarial vaccine research. At the same time, the introduction of high-throughput technologies enable examination of the entire molecular repertoires of both parasite and hosts. Combined with application of system analyses these data are being used to identify the pathways that induce and regulate protective immunity. Furthermore, this combination also identifies traits that can lead to pathology, and the evolutionary and ecological forces driving potential vaccine failures. Our best hopes may lie with designing vaccines that do not exist naturally - i. e. genetically modified antigens, and adjuvants that decouple immunopathology from protective immune responses.

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**Table 1**

Examples of protective immunity in evoked by selected filarial recombinant proteins and/or plasmids carrying filarial antigen genes alone or as multivalent formulations.

Antigen	Delivery	Host	Protection	Assessment	Predominant Response(s)	Reference
<i>Brugia malayi</i> BmVAL-1 vespid venom allergen homolog-like protein (also described as VAH)	DNA	Mice	39%	L3 in IP chambers	IgG1, IgG2a IFN- $\gamma$ , IL5	(79)
BmVAL-1	Protein	Mice	48%	L3 in IP chambers		(79)
BmVAL-1	DNA + protein boost	Mice	54%	L3 in IP chambers		(79)
BmVAL-1	DNA	Jirds	50%	Adult worms		(79)
BmVAL-1	Protein	Jirds	40%	Adult worms		(79)
BmVAL-1	DNA + protein boost	Jirds	52%	Adult worms		(79)
BmALT-2 (abundant larval transcript)	DNA	Mice	51%	L3 in IP chambers	IgG1, IgG3	(79)
BmALT-2	Protein	Mice	65%	L3 in IP chambers		(79)
BmALT-2 + Bm VAL1	DNA + Protein boost	Mice	74%	L3 in IP chambers		(79)
BmALT-2	DNA	Jirds	58%	Adult worms		(79)
BmALT-2	Protein	Jirds	72%	Adult worms		(79)
BmALT-2	DNA + Protein boost	Jirds	78%	Adult worms		(79)
BmALT-2 + Bm VAL1 (prime and boost)	DNA + protein boost	Mice	82%	L3 in IP chambers	IgG1, IgG2a, IgG3	(79)
BmALT-2 + Bm VAL1 (prime and boost)	DNA + protein boost	Jirds	85%	Adult worms		(79)
BmALT-2 + BmVAH	DNA	Jirds	57%	Adult worms	IgG2a, IgG2b	(7)
BmALT-2 + BmVAH	Protein	Jirds	80%	Adult worms	IgG1, IgG2a, IgG2b, IgG3	(7)
BmALT-2	Protein	Jirds	70%	L3 in IP chambers	IgG1, IgG3	(7)
BmVAH	Protein	Jirds	60%	L3 in IP chambers	IgG2a	(7)
BmALT-2	DNA	Mice	34%	L3 in IP chambers	IgG2a, IgG2b, IgA	(107)
BmALT-2	Protein	Jirds	75%	Adult worms	IgG1 IL4, IL5	(107)
BmALT-2	DNA	Jirds	57%	Adult worms	IgG2a, IFN-g	(107)
BmALT-2	DNA + protein boost	Jirds	64%	Adult worms	Mixed Th1 Th2	(107)
Bm TPX Thioredoxin peroxidase	DNA	Mice	37%	L3 in IP chambers	IgG2a, IgG2b, IgA	(108)
BmALT-2 and thioredoxin peroxidase (TPX)	DNA	Mice	78%	L3 in IP chambers	IgG2a, IgG2b, IgA	(108)
<i>O. volvulus</i> Glutathione-S transferase (GST)	Protein	Jirds	82.75%	Adult worms	Mixed Th1 Th2 as assessed in mice immunised with recombinant protein	(109)
OV-FBA-1 fructose-1,6-bisphosphate aldolase	Protein	Mice	50%	L3 in IP chambers	nd	(110)

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Recombinant DNA vaccines have been administered by subcutaneous (sc) injection. Inoculation with recombinant proteins has also been by the sc route. DNA refers to recombinant DNA plasmids containing the filarial antigen gene sequence. Protein refers to recombinant filarial antigen. Assessment of protective immunity in the mice has been by counting the number of surviving L3 larvae contained in millipore chambers that may measure up to 14mm diameter and 2mm depth (variation will occur) and which have been surgically implanted into sub-cutaneous or tissue or into the peritoneal cavity. In the jird (*Meriones unguiculatus*) model of *B malayi*, adult worm burden is used to assess efficacy of vaccination. Levels of protection are expressed as a percentage recovery or reduction in parasite burden of vaccinated animals versus unvaccinated control animals. For additional information on *Onchocerca* vaccine trials see (80).