Filarial Immunomodulatory Strategy as a Treatment against Diseases

Subjects: Parasitology

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Lymphatic filariasis is an infection in humans caused by filarial parasites: *Wuchereria bancrofti*, *Brugia malayi*, and *B. timori*. To ensure effective transmission, these parasites evolved with multiple hosts, including a human as a definitive host and the mosquito as an intermediate host. Targeting filarial immunomodulators and manipulating the filariae-driven immune system against the filariae can be a potential therapeutic and prophylactic strategy.

Keywords: lymphatic filariasis ; inflammatory diseases ; filariae

1. Lymphatic Filariasis

In 1997, the World Health Assembly set the goal of eliminating lymphatic filariasis globally by 2020 through mass drug administration (MDA). During MDA, all individuals living in endemic areas received one of these single-dose two-drug combinations: albendazole (ALB) + diethylcarbamazine (DEC) citrate; ALB + ivermectin (IVM) in areas co-endemic for onchocerciasis; or ALB, preferably twice a year, in areas co-endemic for loiasis. However, numerous obstacles stand in the way of successful implementation. These drugs are only effective against microfilariae and not against adult and larval parasites. By the end of 2020, MDA had not yet been delivered to ten endemic countries [1], which raised concerns about the recurrence of filarial infections in countries or areas that were previously declared free of LF infection [2]. One reason for this concern is human migration from endemic to LF-free areas [3][4][5][6]. The majority of migrants were from rural endemic areas, which had poor sanitation, rice fields, and inadequate mosquito control. Moreover, climate change and delays in MDA due to COVID-19 are likely to further sabotage eradication efforts [7][8]. According to the WHO's 2021 report, 859 million people in 50 countries are at risk of lymphatic filariasis, which requires preventive treatment. As a result, the WHO revised the target date to 2030, using a triple-drug MDA combination of IVM, DEC citrate, and ALB (IDA-MDA), which may result in patient non-compliance [9][10][11][12]. This evidence demands the development of effective vaccines and novel therapeutics.

Strikingly, current antifilarial drugs target the immunomodulatory arsenal of filariae; they alter the host-parasite interface, unmasking the host immune system to access the parasite. The widely used drug DEC is believed to block PGI2 and PGE2 production in both microfilariae and endothelial cells. The resulting vasoconstriction enhances endothelial adhesion and microfilariae immobilization as well as destruction by host platelets and granulocytes [13]. IVM prevents protein release from microfilarial extracellular vesicles by blocking the GluCl channel. These proteins are indispensable for evading the host immune system [14]. Maclean et al. (2021) recently investigated the effects of DEC and IVM treatment on the *B. malayi* gene expression that may be responsible for filarial clearance from blood circulation [15]. For example, treatment with either IVM or DEC downregulated galectin expression in adults. Galectins, among many other immunomodulatory effects, impede lymphocyte trafficking [16], stimulate alternative macrophage activation [17], and cause T cell apoptosis [18]. Since oxidative and xenobiotic detoxification mediated by antioxidants is a fundamental survival strategy for filariae, researchers synthesized and studied the library of sulphonamide chalcones that affect filarial GSH status, produce oxidative stress, and lead to apoptosis [19][20].

Indeed, drugs can heal existing infections, but they will not prevent infections unless they, or their active metabolites, are removed slowly from the host system, and remain in circulation for a lengthy period. Given that filariae orchestrate the host's immune system for their own growth and survival, manipulating the host's defense system against LF could be a viable prophylactic option. Several potential vaccine candidates have been identified and tested for their potential against LF [21]. Many antigens are non-homologous to human and immunomodulatory proteins that subvert the host's immune response against the parasite.

Table 1 summarizes immune-regulatory proteins that have been evaluated as vaccine candidates. *B. malayi* immunomodulatory proteins such as heat shock protein 12.6 (BmHsp12.6αc), abundant larval transcript-2 (Bm-

ALT-2), and tetraspanin large extracellular loop (Bm-TSP LEL), showed maximum protection in mouse challenge experiments [22][23][24]. To improve the protective efficacy of monovalent vaccines, these best vaccine candidates were fused to prepare a single multivalent vaccine, rBmHAT (BmHsp12.6 + BmALT-2 + BmTSPLEL). Strikingly, it showed >95% protection against *B. malayi* infection in mice when AL007 or AL019 was used as an adjuvant [25]. However, when administered with alum in non-human primates, rBmHAT provided ~35% protection [26], hinting at a need to change the adjuvant and/or multivalent formulation before using this vaccine in human clinical trials. Adding another immunomodulatory antigen, thioredoxin peroxide (BmTPX-2), to rBmHAT showed >88% protection against the challenge infection [27]. This tetravalent rBmHAXT confers approximately 57% protection against challenge infections in a primate model, which meets the WHO requirement, and hence offers great potential for using this vaccine in human clinical trials [28].

Table 1. Filarial immune-regulatory proteins that have been evaluated as vaccine candidates.

Candidate Vaccine	Outcome	Reference
Serpin (BmSPN-2)	**Immune response is strong but short-lived, suggesting that serpins alone are not effective vaccine candidates for long-term immunity**	[29]
Aabundant larval transcript-2 (BmALT-2)	**BmALT-2 protein and Bm-alt-2 DNA conferred approximately 75% and 57% protection, respectively**	[23]
Glutathione-S-transferases (WbGST)	**61% protection in Jirds challenge experiments and 65.5% protection in in situ challenge studies**	[22][30][31]
Small heat shock protein HSP12.6 (BmHsp12.6αc subunit)	**83% protection in mouse in situ challenge studies**	
Large extracellular loop of tetraspanin (BmTSP-LEL)	**64% protection in mouse in situ challenge studies**	[24]
Bivalent vaccines: HSP12.6 + ALT-2, HSP12.6 + TSP-LEL, TSP-LEL + ALT-2	**90%, 80%, and 82% protection in mouse in situ challenge studies, respectively**	[32]
Trehalose-6-phosphate phosphatase (BmTPP)	**78.4% decrease in microfilariae counts and 71% reduction in adult parasite load in Mastomys**	[33]
Thioredoxin (WbTRX), Thioredoxin peroxidase (WbTPX)	**57% and 62% protection in Mastomys challenge experiments, respectively**	[34]
BmHAT Trivalent vaccine	**Protein and DNA protein prime boost vaccination yielded approximately 95% protection in mice 3 out of 5 vaccinated macaques were protected from challenge infection**	[26][32]
Cystatin-2 in which the amino acid Asn66 was mutated to Lys66 (Bm-CPI-2M)	**48.6% and 48.0% at 42 and 90 days post-infection, respectively, with *B. malayi* L3 filariae**	[35]
BmALT-2 with Tuftsin as fusion protein	**65% larvicidal activity in ADCC experiments**	[36]
Calreticulin (BmCRT)	**Offers protection during experimental lymphatic filariasis**	[37]
BmHAXT Tetravalent vaccine	**88% protection in mouse in situ challenge studies 57% protection in rhesus macaques challenge infections Reduced fecundity and adult worm burden in Jirds**	[27][28][38]

Abbreviations: BmHAT refers to BmHsp12.6 + BmALT-2 + BmTSPLEL; and BmHAXT refers to BmHsp12.6 + BmALT-2 + BmTSPLEL + BmTPX-2.

2. Malaria

Co-infections are common in endemic regions. Control of intracellular pathogens, such as *Plasmodium* species that cause malaria, *Leishmania donovani*, *Mycobacterium tuberculosis* (Mtb), and human immunodeficiency virus (HIV), requires pro-inflammatory Th1 (IL-12, IFN-γ, and TNF-α) and Th17 (IL-17A and IL-23) responses. Increasing evidence suggests that filariae-driven Th2 and Treg immunity can negatively affect the host's ability to combat these pathogens.

The effect of filarial co-infection on *Plasmodium* spp. has already been discussed in detail [39]. Human and animal studies on LF/malaria co-infection have provided conflicting results, with some demonstrating more severe malaria in the presence of filarial co-infections and others suggesting filariae-induced protection against malaria, depending on the infection severity and parasite type [40][41][42][43][44][45]. A strong Th1 immune response plays a major role in controlling

primary malaria infection. However, the filariae-induced IL-10-dependent Th2 immune response modulates inflammatory IL-12p70/ IFN-γ pathways and increases resistance to malaria [43]. Moreover, pre-existing filarial infection can impair the immunogenicity of anti-Plasmodium vaccination, as evidenced by decreases in plasmodium antigen-specific $CD8^+$ T cells, IFN-γ, and TNF-α production, resulting in reduced cytotoxicity and protection against malarial infection [46]. A simple solution to filarial interference with vaccination efficacy is deworming before vaccination [47]. However, there are several obstacles to drug-induced abolition of filarial infection in endemic locations. These include (1) the lack of an adulticidal or adult-sterilizing drug or vaccine, (2) the time it takes to return to a normal immune response, and (3) the risk of re-infection during the recovery period. Therefore, it is desirable to optimize appropriate vaccination regimes that elicit a multifaceted and potent immune response in filariae-infected individuals [46][47].

Unlike acute malaria, cerebral malaria and malarial sepsis are triggered due to exaggerated pro-inflammatory responses; filariae-derived immunosuppression can protect against this severe immunopathology [40][44]. However, maintaining a filarial infection to avoid an inflammatory exacerbation is not a smart option. In-depth study is required to strike a delicate balance between permissive filarial infection that does not progress to lymphatic filariasis and appropriate immunosuppression that does not lead to severe complications of malaria. Therapies that imitate filariae-derived immunosuppression may be investigated for the treatment of cerebral malaria.

3. Leishmaniasis

Leishmaniasis, the third most common vector-borne disease after malaria and lymphatic filariasis, is caused by the protozoan *Leishmania* parasite. Visceral leishmaniasis, also known as kala-azar, is caused by *L. donovani* and *L. infantum* throughout Asia, North Africa, Latin America, and Southern Europe. Every year, 700,000 to 1 million new cases are reported. The WHO actively encourages research into effective leishmaniasis control [1]. Fractions derived from *B. malayi* were found to cross-react with sera from hamsters infected with *L. donovani*, suggesting that these filarial cross-reactive molecules can contribute to the development of anti-leishmanial prophylactics [48]. In vivo studies in hamsters demonstrated that *B. malayi* L3/adult worms or immunization with a fraction of the adult parasite extract (BmAFII) inhibited the progression of both filarial and *L. donovani* infections [49][50]. Recently, studies have shown that heat shock protein 60 (BmHSP60) shares several antigenic regions of B- and T-cell epitopes of leishmania counterparts and protects against leishmanial infection via Th1-mediated immune responses and NO production [48][51]. In contrast, a fraction of *L. donovani* (Ld1) that cross-reacted with sera of *B. malayi* infected animals facilitated filarial infection. Ld1 consists of eight proteins, including HSPs [52]. Therefore, more comprehensive and in-depth investigations are needed to optimize and develop prophylactics based on cross-reactive rationale in co-endemic regions.

The prevalence of filarial and leishmanial co-infections has been reported in some parts of the world [53]. In the co-infected mouse model, local immune responses to filarial and leishmanial infections were polarized and compartmentalized [54]. These findings contradict acute malarial findings in which microfilariae and *Plasmodium* share the same niche—blood. In popliteal lymph nodes (which drain the *L. major* infection site) and thoracic lymph nodes (which drain the *L. sigmodontis* infection site) immune responses were IFN-γ- and IL-4-dominant, respectively. Moreover, pre-existing helminth infection delayed IFN-γ production and *L. major*-induced lesion progression [54]. Notably, unlike the leishmanial co-infection model, which confines parasites to the thoracic cavity, the presence of human lymphatic microfilariae in the bloodstream may provide a different immune outcome. Appropriate filarial animal and human population studies are needed to assess whether the immune response to LF/leishmaniasis co-infection is defensive or progressive; such assessment will aid in the development of appropriate and specific immune modulation therapies.

4. Inflammatory Diseases

In developed societies, large-scale deworming programs, reduced exposure to infection due to vaccination, and improved sanitation are associated with an increase in the occurrence of inflammatory and metabolic disorders, supporting the hygiene hypothesis [55][56][57]. The ability of parasitic worms to shift the immune response from Th1 to Th2/Treg has sparked interest in employing live worms as immunotherapy. However, rather than reintroducing an infection, one approach to reducing the incidence of inflammatory and autoimmune disorders is to employ substitutes for these infections that retain their protective benefits.

Many studies regarding the therapeutic potential of helminthic proteins in inflammatory diseases have recently been discussed [58][59]. The use of non-human helminthic proteins may be one reason for unsuccessful clinical trials. Human filariae have co-evolved with the human immune system, suggesting that it is more suitable to use human filarial proteins over other helminthic proteins. There is strong evidence in mouse models that human filarial therapy, excretory-secretory components, and their recombinant molecules can treat and/or prevent inflammatory diseases such as inflammatory

bowel disease (IBD), type-1-diabetes (T1D), and rheumatoid arthritis (RA) (**Table 2**). However, potential filarial proteins have only been tested in the laboratory and have not been tested in clinical trials. Effective coordination can reduce duplication of work, as many proteins have the same mode of action in different inflammatory diseases. For example, rBmALT-2 has been found to reduce the severity of T1D and IBD by downregulating IFN-γ and upregulating IL-10 and IgG1/IgG2a [60][61]. Although promising results have been achieved with human lymphatic filarial therapy, many questions, such as those regarding optimal dose, treatment duration, immunization route, safety profile, and cellular mode of action, remain unanswered. There is considerable scope for research in this area. For instance, site-directed administration of filarial immunomodulatory proteins using anti-colitic probiotics can provide effective IBD prevention/cure therapy [62]. A series of research studies, ranging from basic to clinical, is essential to evaluate the efficacy, safety, tolerability, and ethical implications of genetically modified immunobiotics.

Table 2. Human lymphatic filariae-derived molecules as a therapy against inflammatory diseases.

Lymphatic Filarial Protein	Experimental Disease Model	Study Outcome	Mechanism of Action	Reference
Recombinant *B. malayi* Cystatin (rBmCys)	DSS-induced acute colitis	Down-regulated inflammatory responses and alleviated symptoms and pathology of colitis.	Elevated IL-10 + FoxP3 + Tregs, IgM + B1a cells and AAMs in the colon and peritoneal cavity. Reduced expression of Th1 and Th17 cytokines in serum and spleen.	[63][64]
rBmCys	mBSA-induced rheumatoid arthritis (RA)	Both preventive and therapeutic effects on RA. Decreased synovitis, bone erosion, fibrosis, and influx of inflammatory cells in hind paw joints.	Shift from Th1 to IL-4 and IL-10 secreting Th2 immune response.	[65][66]
Peptide fragments of rBmCys	DSS-induced acute colitis	Anti-inflammatory effect on DSS-induced colitis in mice. Reversed the gross and histopathological changes in the colitic colon.	Decreased F4/80 + TLR-4 + CD11c+ macrophages in peritoneum. Reduced LY6G+ cells and MPO+ cells and increased FoxP3 + Tregs in colon.	[67]
Recombinant *B. malayi* abundant larval transcript-2 (rBmALT-2)	DSS-induced acute colitis	More effective in preventive mode compared to therapeutic treatment against colitis.	Associated with downregulation of IFN-γ, IL-6, IL-17, and upregulation of IL-10 cytokines in spleen.	[60]
Recombinant *W. bancrofti* L-2 (rWbL2)	DSS-induced acute colitis	Reduced lymphocyte infiltration and decreased epithelial damage in colons of treated mice.	Shift towards Th2 response as reflected by increased IL-10, and decreased IFN-γ and TNF-α by splenocytes. IgG1/IgG2 ratio in the sera.	[68]
rBmALT-2, rBmCys, and rWbL-2 individually and in combinations	DSS-induced chronic colitis	All treatment strategies improved the clinicopathologic status of chronic colitis. rBmALT-2 + rBmCys showed the most prominent therapeutic effect.	Downregulated IFN-γ and TNF-α expression, upregulated IL-10, and TGF-β expression in the splenocytes. Reduction in activated NF-κB level in the colon. Increased IgG1/IgG2 ratio in the sera.	[69]
rWbL-2, rBmALT-2, and rWbL-2 + rBmALT-2	STZ-induced T1D	Led to reduced lymphocytic infiltration, islet damage, and blood glucose levels.	Decreased TNF-α and IFN-γ, and increased Il-4, IL-5, and IL-10 production in splenocytes. Elevated insulin-specific IgG1 and antigen-specific IgE antibodies in the sera.	[61]
B. malayi adult soluble (Bm A S) and microfilarial excretory-secretory proteins (Bm Mf ES)	STZ-induced T1D	More effective when used as curative rather than a preventive treatment. Reduced inflammatory changes in pancreatic islet cell architecture and fasting blood glucose levels.	Decreased TNF-α and IFN-γ, and increased IL-10 production in the splenocytes. Elevated anti-insulin IgG1 antibodies indicating a skewed response towards Th2 type in the sera.	[70]

Lymphatic Filarial Protein	Experimental Disease Model	Study Outcome	Mechanism of Action	Reference
***B. malayi* asparaginyl-tRNA synthetase (BmAsnRS)**	**T-cell transfer colitis**	**Resolves intestinal inflammation.**	**Increase in $CD8^+$ T cells in the lamina propria compartment, with a corresponding increase in $CD4^+$ cells in spleens of treated mice. Decrease in IFN-γ and IL-17, and increase in IL-4 and IL-10 in spleens, mesenteric lymph nodes, and lamina propria of treated mice. Induced upregulation of IL-10 and IL-22 receptors.**	[71]
***Brugia malayi* K1 (BmK1)**	**-**	**Inhibits the delayed-type hypersensitivity response.**	**Blocked Kv1.3 receptors in human T cells. Suppressed the proliferation of rat CCR7-effector memory T cells and production of IFN-γ.**	[72]

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