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HOST PROTECTIVE IMMUNITY AND VACCINE DEVELOPMENT STUDIES IN LYMPHATIC FILARIASIS

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ABSTRACT

Lymphatic filariasis caused mainly by infection from *Wuchereria bancrofti* and *Brugia malayi* remains as the major cause of clinical morbidity in tropical and sub-tropical countries. Development of vaccine against filarial infection can act as additional measure to the existing therapeutic and vector control methods in the control of this disease. The main hurdles in the development of anti-filarial vaccine are the strict primate specificity of *Wuchereria bancrofti*, the paucity of parasite material, the diversity of clinical manifestations and their associated complex immune responses, lack of clear understanding on host-parasite interactions and the mechanisms involved in protective immunity. However in the past few years, the information generated in immuno-epidemiological studies, correlated with observations in experimental animals suggests that a filarial vaccine is feasible. Initially live irradiated infective larvae have been successfully used to induce high level of protective immunity in several animal models. Applying diverse strategies, variety of purified or recombinant filarial antigens have been explored for their ability to induce protection in different host-parasite systems. Some of these targeted filarial antigens induced high level of resistance in experimental animals against challenge infections. More focussed studies on thorough characterization of parasitological and immunological changes associated with resistance induced by such candidate protective antigens and on delivery mechanisms and safety aspects will be crucial in their selection for possible use in humans.

KEY WORDS: Lymphatic filariasis, *Wuchereria bancrofti*, Brugia malayi, protective immunity, immunoprophylaxis, vaccine.

FILARIAL PARASITE & DISEASE

Filariasis is a chronic debilitating disease caused by nematode parasites of the order 'Filaridea' commonly called 'Filariae'. Of different types of filarial infections, lymphatic filariasis caused by *Wuchereria bancrofti* and *Brugia malayi* is prevalent in rural and urban slums of many tropical countries, predominantly affecting the poorer sector of the community. Although, almost never directly fatal, chronic infection can lead to disability, disfigurement causing untold pain, misery and impairment of health. Recent studies on socio-economic impact of the disease showed that its manifestations inflict immense social, psychological and economic burden on affected individuals and their families (1).

Filarial parasites are obligate parasites with complex life cycles. The cycle includes an essential molting stage, in an intermediate host mosquito (12-14 days) and a period of further development followed by reproductive activity in the definitive host, man. The infective larvae enter skin through mosquito bite wound, migrate to the lymphatics of the host and mature into adults in 6-12 months. The fertilized female produces a number of microfilariae (mf), which are picked up by the female mosquito while it feeds, and further transmission continues in the community (2).

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Lymphatic filariasis is characterized by a wide spectrum of clinical manifestations in individuals of endemic region, with signs and symptoms often differing from one endemic area to another (3,4). The type of clinical presentation is often the outcome of a complex interaction of several factors and processes including the human immune response, the varying stages of parasite and their molecules and the environment. The spectrum of manifestations include (a)asymptomatic microfilaraemia (having mf in circulation but without any clinical manifestations); (b) acute filariasis (which generally includes fever with chills, rigor, pain and swelling of upper and lower limbs associated with lymphangitis and lymphadenitis and genital manifestations like epididymoorchitis and funiculitis in male); (c) chronic manifestations (that mainly include lymphoedema, hydrocele and elephantiasis) and d) occult filariasis (with out mf and having atypical manifestations like tropical pulmonary eosinophilia (TPE), arthritis, etc.,). Apart from these, any endemic population comprises of a certain group, often described as 'endemic normal' having no detectable mf and without any symptoms. It is possible that some of these people have sub-threshold microfilariae or sub-clinical infection (5) and some may be 'truely' immune to the parasite.

IMMUNOLOGICAL APPROACH TO CONTROL FILARIASIS

A rational immunological approach in the control of lymphatic filariasis has been a challenging problem due to the limited understanding of biology and immunobiology of these unique parasites. The other main problems that hindered the development of filarial vaccine are the strict primate specificity of *W. bancrofti*, the paucity of parasite material for identification of protective antigens and the diversity of clinical manifestations and their associated complex immune responses. There has been no sufficient data available on mechanisms of host-parasite interactions and on the phenomena responsible for protective immunity. Inspite of these limitations, the varied strategies being employed for the development of filarial vaccine have resulted in a significant progress in this direction in the recent years (6,7).

PROTECTIVE IMMUNITY IN FILARIASIS

A) Acquired protective immunity

Immunity to filarial infection has generally been defined as the absence of evidence of infection or sign of disease in an individual who is exposed to continuing parasite transmission. Although the proof of such sterile immunity against filarial infection is not conclusive, it seems that some level of naturally acquired protection does exist (6). This is evident from certain immunological correlates found in the filarial 'endemic normal' group. Both cellular and humoral responses to filarial antigens were found to be higher in this group compared to microfilaraemic cases with or without symptoms (8,9). This heterogenous group with the absence of filarial infection or the presence of infection below the detection threshold is equally exposed to the parasite as the other infected groups. Such 'putatively immune' sub population in a filarial endemic area provides an indirect evidence of presence of naturally acquired protective immunity in humans against filarial infection (10,11). The existence of this putatively immune group presents a strategy for the identification of antigen(s) that might provide host protection upon prophylactic immunization (10). The antigens preferentially recognized by this group and not by microfilaraemic cases can be considered as promising candidates for prophylactic use.

B) Concomitant immunity in filariasis

Immunity, which protects the already infected individuals from super infection with further waves of parasites, is termed as 'concomitant immunity'. Here the protective immune response is specifically targeted against infective larvae while the already residing fecund adult worms remain functionally intact. In an immuno-epidemiological study in bancroftian filarial endemic area of Papua New Guinea (PNG), Day et. al. (12) showed that there were no any 'worm-free' endemic normals in that population with intense filarial transmission. Based on circulating levels of phosphoryl-choline containing antigen (considered as a marker of active infection) it has been suggested that amicrofilaraemic individuals are likely to have same adult worm burden

as low microfilaraemic cases and their status of being amicrofilaraemic is possibly due to concomitant immunity.

In longitudinal studies on filarial infection dynamics in PNG (12) and Pondicherry, India (13), it was observed that the rate of gain of infection reached peak at the age of around 20 years and thereafter remained constant or reduced. These vital field data support the existence of concomitant immunity directed against early larval stage acquired by already infected adults with increasing exposure to infection.

The findings on the 'concomitant immunity' in humans can be correlated with observations in experimental animals where it has been shown that the resistance to filarial infection can be induced followed by repeated inoculations with infective larvae. Administration of multiple doses of varying or similar numbered infective larvae called as 'trickle infections' generally induces strong resistance against different filarial parasites in animals. After trickle infections the challenge larvae get killed leading to eventual reduction or total clearance of adult worms. Such experimental induction of concomitant immunity was demonstrated in *Brugia pahangi* infected cats (14,15), *Acanthocheilonema viteae* infected hamsters and jirds (16) and *W. bancrofti* infected mice (17). These findings in epidemiological and laboratory based research on the development of concomitant immunity suggest that infective larval antigens may be of paramount importance in induction of acquired immunity to filarial infection (11). The fact that such protective immunity against early larval stages can develop inspite of the existence of immunological tolerance to other stages (adult and/or mf) suggests that the antilarval immunity is stage specific and different from immunity against other stages of parasite (18). Induction of protective immunity against infective larval antigens may not be pathogenic and this implies that infective larval antigens are safe protective immunogens to look for as candidate vaccines for filariasis.

VACCINE DEVELOPMENT STUDIES IN LYMPHATIC FILARIASIS

A) Animal models as aids in vaccine research:

There is no established and convenient animal model for the major lymphatic filarial parasite *W. bancrofti*. Attempts to obtain full development of *W. bancrofti* even in congenitally athymic (nude) mice and immune suppressed gerbils were not successful (19). *W. bancrofti* will only reach reproductive immunity in primates i.e. leaf monkeys (20). As such modeling of lymphatic filariasis is complicated by the diversity of infection in natural host, human. No single inbred species of laboratory animal mimics such a spectrum of susceptibilities and antipodal immunological correlates (21) for which not only environmental (22) but also genetic causes (23) have been implicated. Hence natural mammalian hosts for non-human filarial parasites (e.g. dog for *Dirofilaria immits* or cat for *B. pahangi* and *A. viteae* in jirds) or surrogate hosts with varying permissiveness like those of fully permissive (e.g. jirds, cats, nude mice for *Brugia*), semi permissive (BALB /c mice) and non-permissive have been explored to identify the protective immunogens and study the mechanisms involved. In these studies animals are immunized with filarial parasites or their antigens and the immune response generated is analyzed for anti-parasite effects. The sera collected from immune animals are tested in vitro to induce antibody-dependent cell-mediated cytotoxicity (ADCC) against the parasite in the presence of effector cells. Implantation in immunized animals of micropore chambers constructed of inert plexiglass rings, sealed with nucleopore membranes and loaded with larval parasites is another useful technique to evaluate the acquired immunity. The recovery of adult worms or L_4 larvae from immunized animals followed by challenge infective larval infections can provide information on the protective effect of the immunogen.

B) Immunoprophylactic studies using whole parasite

In the early experiments, live irradiated third stage (infective) larvae or naive larvae have been used to induce high level of protective immunity in several

animal models. The first such attempt was made in 1969 by Wong et. al. (24) using sub-periodic *B. malayi* in monkeys. Since then successful results using irradiated L3 larvae as vaccines have been reported in many host parasite systems, including *B. pahangi* in dogs (25), cats (26), jirds (27) and mice (28), *Brugia malayi* in jirds (29) and mice (30, 31) and *Litomosoides carini* in rats (32). Repeated exposure to infective larvae followed by termination of infections using anti-filarial drugs has also been shown to effectively stimulate the host immune system and induce protective immunity against challenge infections (33).

C) Immunoprophylactic studies using parasite antigens

Several types of crude and purified filarial antigens have been explored as protective immunogens. These include adult or larval extracts, antigens on parasite surface, excretory-secretory (ES) products and functional molecules (e.g. enzymes) essential for the growth and development of parasite in the host. The parasite antigens preferentially recognized by putatively immune endemic normals and not by infected microfilaraemic cases have also been tested to induce protection in experimental animals.

Immunization of animals with microfilarial or adult worm extracts (34,35) and infective larval antigens (36,37) has been shown to induce resistance against infective larvae. Immunization of jirds with a water soluble extract of *B. malayi* mf enhanced the host's ability to eliminate adult worms and blood-borne mf (38). In earlier studies in our laboratory *B. malayi* mf and infective larvae ES antigens have been shown to induce ADCC reaction both *in vitro* and *in situ* (micropore chamber technique) against mf and L3 larval stages of the parasite (39).

In another report from this laboratory two purified filarial antigens i.e. a 120 kDa Bm A-2 of *B. malayi* adult worms and a 43 kDa circulating filarial antigen fraction-2 (CFA-2) of microfilaraemic plasma were found to be highly reactive with endemic normal group (40). Both these antigens were further explored for their immunoprophylactic activity against *B. malayi* infection in jird model. Sera collected from Bm A-2 immunized jirds induced a significant level of protection against mf and L3 larvae in *in vitro* ADCC and *in situ* micropore chamber methods (41). Immunization of jirds with BmA-2 antigen resulted in 88% reduction in the development of parasite to adult stage (Fig.1).

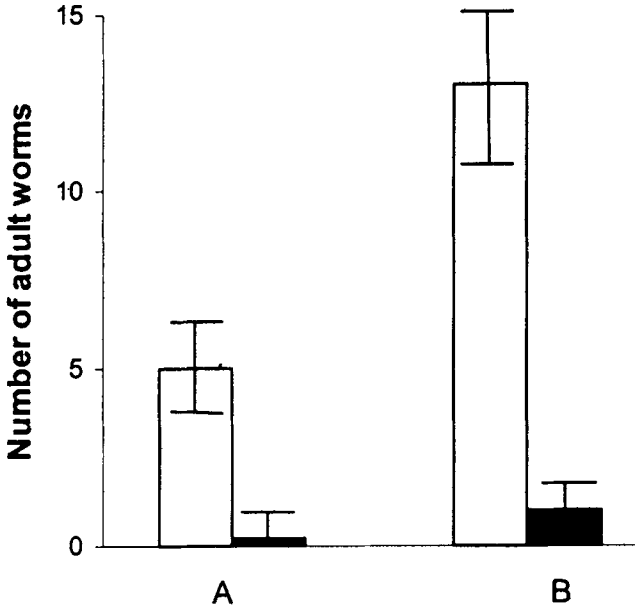

Figure 1. Reduction in the number of adult worm recovered from the control (□) and immunized (■) jirds inoculated with 50 (A) or 100(B) L_3 larvae (each value is the mean ± SD of the results obtained from four individual jirds) (Source: Reference No. 41).

Passive transfer of immune sera from jirds immunized with BmA-2 to naive jirds resulted in 71% reduction in adult worm recovery as observed 90 days after challenge infection with *B. malayi*. On the other hand the passive transfer of non-adherent spleen cells from immune jirds did not show any significant effect on the development of parasite (Fig.2) suggesting importance of humoral immunity in protection. Antibody dependent cellular cytotoxicity using chronic filarial serum has been demonstrated earlier to explain amicrofilaraemia in clinical cases (42).

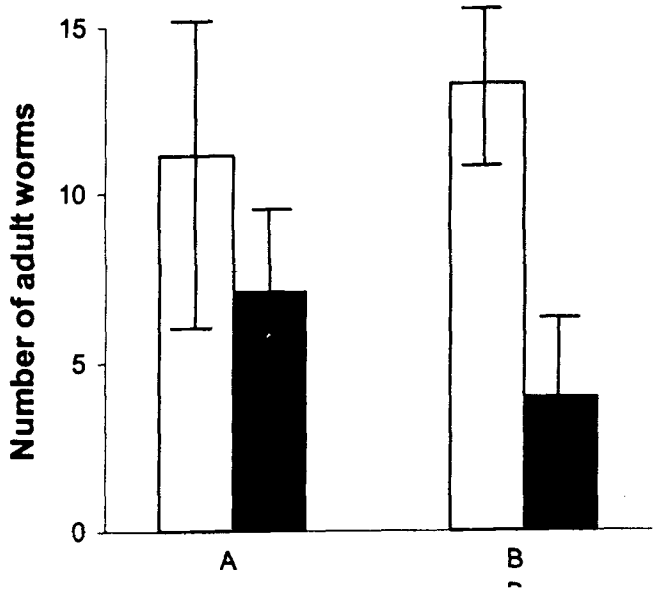

Figure 2. Enhanced immunity against the development of parasite in jirds transferred with T cells (A) and sera (B) from immunized animals (■) as compared ;with normal animals (□) (each value is the mean ± SD of the results obtained from three individual jirds) (Source: Reference No. 41).

In another study (43) a strong protective response of approximately 84% was observed against the development of filarial parasite in the jirds immunized with CFA2-6 (Table1). In this study the immunized jirds also showed a significant clearance (87%) of microfilariae inoculated intravenously. Both the antigens i.e. CFA2-6 and Bm A-2 were also found to be cross reactive with each other. However CFA2-6 also showed a different pattern of cross-reactivity with other filarial antigens suggesting the presence of different epitopes along with cross-reactive epitopes (43).

Freedman et. al. (10) identified a 43 kDa protein of *B. malayi* selectively recognized by putatively immune endemic normals. Raghavan et. al. (44)

Table 1. Number of adult worms recovered from the jirds immunised and then challenged with *B. malayi* L_3 larvae on the 90th day post-challenge*.

Jirds	Number of Adult Worms		MF Status
	Male	Female	
Test (CFA_2-6)			
1	3	0	-ve
2	2	0	-ve
3	2	0	-ve
4	1	0	-ve
5	0	3	-ve
6	3	1	+ve
7	0	1	-ve
8	1	0	-ve
Mean±S.D.	1.5±1.19	0.65±1.06	
Control (Control antigen)			
1	6	8	+ve
2	9	0	-ve
3	10	5	+ve
4	11	0	-ve
5	10	6	+ve
6	7	6	+ve
7	5	5	+ve
8	9	3	+ve
Mean±S.D.	8.3±2.13	4.12±2.90	
Normal (PBS control)			
1	8	0	-ve
2	4	8	+ve
3	10	3	+ve
4	7	5	+ve
5	13	2	+ve
6	10	8	+ve
7	8	3	+ve
8	8	6	+ve
Mean±S.D.	8.5±2.61	4.37±2.87	

(Source: Reference No. 42)

*CFA_2-6 immune jirds of control jirds were challenged (i.p.) with 100 L_3 larvae 10 days after the last dose of immunization and the adult worms were collected fromthe peritoneal cavity upon necropsy on the 90th day post-challenge.

cloned and characterized this 43 kDa protein as chitinase-like antigen. It was also shown to have sequence conservation with that of the 43 kDa antigen of *W. bancrofti* and 40-60 % sequence homology to bacterial chitinases. Immunization of jirds with recombinant chitinase induced partial protection against microfilaraemia resulting from subsequent infection with *B. malayi* but there was no reduction in adult worm burden (45). Immunization of jirds with another recombinant antigen SXP1, which is present in multiple worm stages also reduced microfilaraemia, but hyper immunization with a recombinant filarial myosin was not protective (45). Li et. al. (46) identified 97, 60, 55 and 10 kDa antigens of *B. malayi* L3 larvae to be reactive with sera of jirds infected with irradiated L3 larvae. The 97 kDa molecule was identified as a muscle protein, paramyosin. Immunization of mice with filarial paramyosin resulted in enhanced clearance of transferred *B. malayi* mf (47). Using the cloned *B. malayi* paramyosin for immunization in jirds 43% reduction in adult worm recovery was observed (48). Recently the same group used plasmid DNA vaccine encoding paramyosin protein in mice and jirds and noted that while strong immune response was generated in the immunized animals, there was no fall in adult worm recovery (49).

In another recent communication by Peralta et. al. (50), three recombinant filarial antigens (parts of filarial heat shock protein, myosin and a-type IV collagen) were identified by screening expression libraries for their differential recognition by endemic normals and microfilaraemic cases. Reporting that none of these immunogens provided protection in susceptible host jird, the investigators aptly suggest that their 'negative' data is consistent with Walkman's conjecture that highly immunogenic parasite antigens may not be appropriate candidates for prophylactic immunization.

Most of the studies reviewed here targeted the infective larvae for immunological destruction based on the available evidence that the immune responses to L3 or L4 larval antigens are unlikely to be pathogenic. Vaccine with L3 or L4 larval antigens is expected to be effective in naïve individuals as well as currently infected cases as an adjunct along with anti-filarial drug therapy . An alternative strategy would be to develop a 'transmission blocking vaccine' by targeting mf stage for immunological clearance. There are indications that antibodies against mf components participate in the clearance of mf in experimental animals and presumably in human (38,39&45). An 'anti-pathology vaccine' may be indicated for the individuals with current infection to prevent the possible development of disease manifestations and an 'immuno-therapeutic vaccine' for the infected individuals where anti-filarial drugs are not effective. Presumably such an intervention will be aimed to sterilize or destroy the adult worms and prevent the generation of disease causing 'agents'.

As more information is being generated on the immunity to filarial infection, varying strategies employed have helped to identify variety of protective antigens in filariasis. The hurdles that have been over come so far in this area should stimulate more focussed research leading to better prospects to develop a filarial vaccine in future.

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