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Immunology of Lymphatic Filariasis: Connecting the dots

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SUMMARY

Immunological studies in filariasis, as in many other infectious diseases, have been undertaken using animal models and comparing them with human disease. A general disconnect in the observations made in these two apparently diverse models have resulted in generating a notion that animal models do not effectively reflect the situation in human filariasis. Notwithstanding the obvious differences between human and animal models of filariasis, it has been possible to identify common grounds that unify the underlying principles of immunology of filariasis. Insight into this has also resulted in clearer understanding of the vexed issue of natural history of human filariasis and has led to the proposal of a model that incorporates existing data from animal models as well as epidemiological and longitudinal observations made in human populations. The model explains the observations and proposes testable predictions.

Key Words: Immunology of Filariasis; Natural history of Filariasis, *W. bancrofti*, Infection and Disease, Btk mice, XID mice, IFN-g, Inflammation.

INTRODUCTION

Filariasis is a spectral disease. Based on parasitological examination of blood for Mf, serological testing for circulating filarial antigens (CFA) and clinical manifestations, the subjects in an endemic area can be classified as i) Parasite carriers: infected subjects who are often free of overt chronic disease manifestations; ii) Chronic pathology: patients who display one or more of the chronic disease manifestations with or often without patent infection; iii) acute disease: subjects who suffer from periodic episodes of acute symptoms characterized by adenolymphangitis and iv) Endemic normals. Typical clinical manifestations, the hallmark of human disease, are seldom observed in animal models of filariasis. If and when comparable clinical features are observed,

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as in *B. pahangi* infected cats, (1) the manifestations are transient, unlike chronic disease in humans which persist for several decades. Development of lymphadema persisting for longer duration has recently been demonstrated in *B. malayi* infected leaf monkeys (2).

A large body of work on human immune responses to all the developmental stages of filarial parasites has been reported from several geographical areas endemic for filariasis such as India, Brazil, Cook Islands, Haiti, Indonesia, PNG etc. (3-6) in both Bancroftian and Brugian Filariasis infected subjects. The immune response phenotypes differ significantly in endemic subjects between those infected with filarial parasites and those free of patent infection. Filarial specific T-cell proliferation, IFN-g, IL-5 and IL-10 production, levels of antibodies to filarial carbohydrates, levels of filarial IgG1, IgG2, IgG4 and presence of anti-sheath antibodies are significantly different between putatively immune subjects in comparison to the infected population. Taken together, these observations indicate that absence of current infection in the host is associated with enhanced Th1 responses and infected hosts display a significantly decreased filarial specific Th1 responses associated with enhanced T-regulatory cell activity resulting in enhanced production of IL-10 and TGF-b. Based on these results it was generally assumed that protective immunity is associated with enhanced Th1 responses in human filariasis. A mathematical model was also proposed on this basis. More detailed discussion on the cytokine balance in the clinical spectrum of human filariasis can be found in a recent review (7). Most of the above observations have been made in case controlled studies. There are a few cohort studies conducted in infected subjects and in endemic controls over a long period of time (8-11). These limited but valuable studies have shown that qualitatively the immune response phenotypes are sustained in both the cohorts over 13-18 years – these investigations have also revealed that immunological hypo-responsiveness associated with patent filarial infections observed in infected subjects persist even after loss of infection.

Rodents such as gerbils and Mastomys couch (multimamate rats) are the most susceptible animal models for human filarial parasites although the precise biological reasons for this are still not understood. However non-availability of crucial immunological reagents and limitations in amenability to genetic manipulations have precluded the use of these animal models to address finer aspects of protective immunity in experimental filariasis. Notwithstanding the limitation that mice are inherently not susceptible to complete development of the human filarial parasite B.malayi, they have been extensively used to study the nature of immune responses to infective larvae and microfilarial stages. Mice are most amenable to study immune responses due to the wide availability of reagents to dissect at the cellular, molecular as well as genetic levels to any antigen/pathogen. In recent years, normal as well as mouse strains with specific genetic deficiencies (gene knock-out mice for specific cytokines, cytokine receptors, immunoglobulins and specific populations of immune cells) have been

used widely to understand protective immunity in filariasis. Several scholarly reviews have been published summarizing these observations (12-14).

The only effective vaccination protocol for helminths, viz., infection with irradiated parasites has also been studied in mice with a view to understand the immune response phenotypes associated with protective immune immunity. Injection of filarial infective larvae or irradiated larvae induce very similar immune responses, viz., a Th2 dominant response that is associated with a down regulated production of Th1 mediating inflammatory cytokines (15-16) suggesting that Th1 responses have a limited role in protection induced by experimental vaccination in mice.

Although immunocompetanat mice are refractory to B.malayi infections, T cell deficient nude mice and IL-5 null mice are relatively more susceptible(12). Interestingly, Brutan's tyrosine kinase (Btk) deficient mice also known as XID mice are susceptible to development of filarial larvae into juvenile adult stage parasites and such mice also do not clear microfilariae in circulation as rapidly as wild type mice. Since a single mutation in one gene made significant differences to filarial susceptibility, extensive investigations were undertaken to study immune responses to filarial infections in XID mice (17-19). Susceptible XID mice exhibited a inflammatory and Th1 polarized immune response to filarial as well as non-filarial antigens, characterized by higher levels of production of TNF-a and IL-1b by their macrophages and higher levels of IFN-g by their T-lymphocytes associated with lower levels of IL-10 in comparison to wild type mice. These observations were juxtaposed with induction of Th2 dominant responses observed in normal wild type mice when injected with filarial larvae as described above (16) It thus emerges that a Th1 polarized immune response is associated with susceptibility to filarial parasites Btk mutant mice while down-regulated Th1 response is a feature observed in refractory immuno-competent mice. Based on this principle four years ago it was then proposed that an inflammatory response is needed for growth and development of filarial larvae while absence of such host responses would render the animals refractory to filarial parasite development (20). Experimental evidence for this proposal has been forthcoming in recent years: - a) Infective larvae of B.malayi (L3) induce primarily pro-inflammatory cytokines (TNF-a and IL-1b etc.,) when put in culture with normal human peripheral mononuclear cells (21) – a similar induction is not observed in PBMCs of microfilariae carriers when exposed to L3s S.Babu, personal communication indicating that non-induction of inflammatory cytokines when challenged with L3s concomitant immunity; could be manifestations as b) induction of Th1 cytokines by pre-injection of CpG nucleotides resulted in enhanced development of filarial larvae into adult worms in jirds (22); c) pre-injection of Carrageenan, a pan macrophage activator of inflammatory cytokines significantly enhanced filarial worm growth and development in both normal as well as nude mice (23) and d) administration of Cyclosporin-A and several other immunosuppressive drugs resulted in

decreased development of filarial worms in experimental animal models (24) ; the above observations strengthen the notion that inflammatory host responses could be needed for larvae development. Taken together these investigations suggest that enhanced Th1 response to filarial antigens is not associated with protective immunity, which appears to be in variance with the broad conclusions drawn in human filariasis as described above. However, a closer and critical appraisal suggests a common unifying basis of immune response in both mice and human if one considers that humans are naturally susceptible to filarial infection while normal mice are essentially resistant. Dissection of immune response phenotype in 'susceptible' and 'resistant' hosts suggests that growth and development of filarial larvae in susceptible hosts including humans are associated with an inflammatory response to filarial parasites (20). Using this central theme the natural history of human filariasis can now be understood and a model is being proposed below.

Very broadly, a "static immunological viewpoint" and a "dynamic model" have been put forward. The static immunological view-point proposes that individuals displaying filarial specific T-cell hyporesponsiveness (down-regulated filarial specific Th1 responses) are associated with development and maturation of filarial worms and such individuals harbor microfilaraemia, while those displaying filarial specific T-cell hyperresponsiveness (upregulated filarial specific Th1 responses) develop pathology and disease and are generally free of patent infection. This implies that differing immune responses predispose individuals either towards harboring infection or developing disease (25). The "dynamic model" proposes that there is a sequential progression from infection, microfilaraemia, and amicrofilaraemia to obstructive disease in all individuals who experience microfilaeraemia (26, 27) and/or that the lymphatic dwelling adult worms essentially mediate pathology and disease (28). Extending this model, it has been proposed that subclinical lymphangiectasia is caused by lymphatic dwelling adult worms and that loss/death of adult worms would result in an inflammatory reaction leading to development of pathology and consequently chronic disease, often assisted by co-factors such as secondary bacterial infections (29). A decade ago, it was also proposed that a breakdown of immunological tolerance associated with patent infection would result in recovery of immunological hyperactivity to filarial antigens and lead to development of pathology and chronic disease (30). While the "immunological view point" was proposed based on immunological read-outs, the dynamic model was proposed on the basis of mathematical derivation using epidemiological data and later by integrating clinical, surgical, ultrasonographic and histopathological data. High prevalence of microfilaraemia and more significantly filarial antigenemia (which detects presence of adult filarial worms in the host, a parameter that did not exist at the time when "immunological view point" was proposed) in elephantiasis and hydrocele patients in several geographical areas do not appear to validate this model

completely (25,31-33). On the other hand, the "dynamic model", proposed about a decade ago, suffers from more serious limitations – presence of patent infection or loss of patency leading to development of disease is central to this model. Since vast majority of patients with chronic disease display immunological hyper-reactivity to filarial antigens, epidemiological proof for the validity of the "dynamic model" is dependant on demonstration of a switch over from the state of immunological hypo-responsiveness (observed during patency) to that of hyper-reactivity and development of chronic disease over a period of time. Longitudinal studies conducted on the same cohort of subjects, the results of which have been reported in recent years, do not offer credence to such a scenario expected of the "dynamic model". Both the models thus continue appear to be limited in their scope (11).

AN ALTERNATIVE MODEL

The different components of an alternative model of progression of filarial infection and disease in naturally exposed human population are shown in Table 1. The model proposed here essentially extrapolates several immunological observations made in experimental animals to development of chronic disease in humans living in endemic areas. In susceptible animal hosts, such as gerbils, dogs, cats, monkeys and chimpanzees, infection with filarial larvae results in inflammatory immune responses followed by down-regulation of such responses, after onset of patency. The pre-patent period in infected animals is consistently associated with an immune response phenotype characterized by enhanced filarial specific T-cell proliferation and release of high levels of IFN-γ by the proliferating T-cells. These characteristic features are "switched-off" once patent infections (with microfilariae/adult worms) set in (20). Extending this sequence of events to infected human populations, the proposed model perceives two stages of parasite development. Stage I, during which the filarial larvae are still developing and are yet to reach maturity and thus the infected hosts do not have circulating filarial antigens. This stage is analogous to the pre-patent period observed in experimental animals. Individuals at Stage I display a hyper-responsive immune phenotype characterized by high levels of filarial specific IgG1, IgG2, IgE and presence of antibodies to Mf sheath. During Stage I, filarial specific lymphocytes proliferate vigorously *in vitro* releasing high levels of IFN-γ and also IL-5. However, production of anti-inflammatory cytokines such as IL-10 and TGF-β are low and levels of filarial specific IgG4 are minimal. The model thus places all subjects with the above features described in the literature (34-38) at Stage I.

Maturation of the developing larvae into adult stage parasites would result in a shift from Stage I to Stage II - a phase in which circulating filarial antigens are detectable; this stage is analogous to the patent phase observed in experimentally infected animals models. The immune response phenotype at Stage II is characterized by lower levels of filarial specific IgG1, IgG2, IgE and absence of antibodies to sheath; proliferation of filarial specific T cells and release of IFN-γ and IL-

5 are also significantly down regulated in this stage. This hypo-responsive phase is characterized by production of high levels of filarial specific IgG4 and release of higher levels of anti-inflammatory cytokines such as IL-10 and TGF-β. The model thus places at Stage II all infected subjects displaying immunological hypo-responsiveness described by several investigators (34-40). The duration of stay at Stage I could vary between individuals in a given endemic area – a few months in some to a few years in others. Several individuals may never move into the patent phase of Stage II. Host as well as parasite factors would contribute in shifting from Stage I to Stage II.

(1) A higher intensity of transmission (greater exposure to infective larvae) would contribute to successful maturation of larvae to adult stage parasites in a larger number of individuals in the area and at a shorter duration of time.

(2) Adult worms and/or their products could offer the required signal for down-regulation of hyper-responsive inflammatory host responses associated with Stage I.

(3) Host genetic factors and/or intra-uterine exposure to filarial antigens/ parasites would predispose the subjects to readily induce immunological hypo-responsiveness that is required for shifting from Stage I to Stage II; and

(4) Presence of intestinal worms in the host could augment filarial worms in down-regulating the inflammatory responses associated with Stage I and assist in establishing patent filarial infections. In general, subjects living in low endemic areas would behave more like experimental animals administered with trickle infections of filarial larvae. Susceptible subjects living in areas of high endemicity and satisfying one or more of the above mentioned predisposing factors would shift more readily from Stage I to Stage II, analogous to susceptible animals reaching patency when infected with a large inoculums of infective larvae.

Histologically, a lymph-node biopsy taken from individuals at Stage I would reveal dead/degenerating worms associated with a severe inflammatory reaction, while those collected from Stage II would have intact, live mature adult worms in dilated lymphatics without inflammatory reaction [41-42]. These are analogous to inflammation and formation of lymph thrombi during pre-patent phase and down regulation of such responses during patent phase in infected animals (43). All Mf carriers and those with cryptic infection (as shown by circulating filarial antigens) are those who have moved into Stage II, while endemic normals are those who have remained stationary at Stage I. A majority of patients with chronic filarial disease, particularly lymphedema/ elephantiasis are those who have remained at Stage I. However, infection pressure above a threshold could down-regulate the inflammatory responses associated with Stage I and shift some of these patients to Stage II, thus accounting for presence of Mf and/or CFA along with chronic symptoms. The relatively higher prevalence of CFA in

patients with hydrocele indicates that shift from Stage I to Stage II takes place more readily in them than in patients with lymphedema/elephantiasis (32,34). The model does not exclude pathogenesis of filarial disease mediated *per se* by lymphatic dwelling adult worms. Parasite-associated factors causing pathology could be operational at Stage II and contribute to the development of disease. Extrapolating from the observations in susceptible animal models of filariasis, the model presumes that a strong inflammatory hyper-responsive state (Stage I) is associated with the growth and development of infective larvae into mature adult worms and that successful persistence of developed worms in the host would depend on rapid down regulation of the inflammatory responses observed in Stage I to an immunologically tolerant Stage II (20,44). The model assumes that the life span of adult filarial worms in infected humans is in the range of 15-20 years, or more. Estimates of the life span of filarial worms are limited to calculations of "fecund life span" only, since they were based on the duration of microfilaraemic phase in Mf carriers (45). Longitudinal follow-up of Mf carriers for 13-16 years has indicated persistence of adult worms as shown by the presence of CFA several years after loss of circulating microfilariae. (10,46). The long life span of adult filarial worms is further indicated by several immunoepidemiological studies on the prevalence of CFA in age-stratified populations in endemic areas. Unlike intestinal worms, which follow a convex prevalence curve (47) filarial antigenemia increases in younger age groups (<20 years) and is maintained as a plateau in higher age groups (34,48,49). Persistence of adult filarial worms for several years (15-20 years or more) would thus maintain the host at the hypo-responsive Stage II. This hypo-responsive state would be irreversible and loss/ death of adult worms would not result in recovery of immunological hyper-reactivity and thus continue to sustain the host at Stage II (50,10). The loss of microfilariae and /or antigenemia does not result in recovery of immunological hypo-responsiveness. Microfilaraemic subjects continue to display decreased filarial specific T-cell proliferation and IFN-γ production and are free of antibodies to microfilarial sheath after loss of circulating Mf/ filarial antigens (50,10). The current model is partly similar to the "Immunological model" in which adult worm infestation is not considered a pre-requisite (unlike the "dynamic model") for development of disease. However, it is unique and clearly different from "immunological view-point" which is essentially bi-directional and considers infection and disease to be generally mutually exclusive. The current model is uni-directional and linear. Secondly, apart from a genetic pre-disposition, it considers infection load/transmission intensity as well as intra-uterine exposure to filarial antigens as crucial components for the consequence and progression of infection/ disease. The model thus accommodates disease development by inflammatory processes (proposed by immunological viewpoint) as well as by lymphatic dwelling parasites, (proposed by dynamic model) and it explains the presence of filarial infection in patients with chronic disease. It also accommodates the observations

made by all the long-term follow-up studies mentioned above (50,10,8,51). Three sets of immuno-epidemiological observations made in endemic areas provide more direct evidence for the major component of the current linear model, which proposes that inflammatory Th1 responses will be observed in the host during the pre-patent phase of infection i.e., before the onset of antigenemia/microfilaraemia. In endemic areas this phase can be expected to be observed under two circumstances – a) in early childhood or b) in transmigrants who have been moved from non-endemic areas to filarial endemic areas. This implies that Stage I features of the model would be observed in younger age groups and relatively more of Stage II features would be observed in the adult population in endemic areas. Existing epidemiological

Table 1: Progression of filarial infection and disease

	Stage-I		Stage-II
Parasite development	L3 → L4 → L5	→	.....L5 → Adults/Mf
Circulating Filarial Antigen (CFA)	Negative	→	Positive
Immune response phenotype	Hyper-responsive state	→	Hypo-responsive state
T-cell proliferation	High		Low
IgG1, IgG2, IgG3	High		Low
IgE	High		Low
IgG4	Low		High
Abs to Mf sheath	Present		Absent
IFN-?	High		Low
IL-5	High		Low
IL-10	Low		High
TGF-?	Low		High
Histopathology	Dead/degenerating worms associated with severe inflammatory reaction	→	Intact, live worms in dilated lymphatics without inflammatory reaction.
Distribution of filarial spectrum			
Mf carriers	0%	→	100%
Cryptic infection	0%	→	100%
Endemic Normals	100%	→	0%
Hydrocele[a]	60%	→	40%
Lymphodema[a] / elephantiasis	80%	→	20%

[a] The prevalence of Hydrocele and lymphoedema at Stage I or Stage II will be variable in different geographical regions depending on transmission intensity.

evidence offer credence to such a possibility (34,52). Transmigrants in Indonesia (who were moved from filarial non-endemic areas to endemic zones) displayed more of Stage I associated immune response phenotype in the early years of exposure (<3 years) and with increasing years of stay in endemic areas many of them moved towards the hypo-responsive Stage II (53). Finally, in Onchocerciasis, early human infection is associated with enhanced parasite specific cellular immune responses, which get down regulated in chronic infections (54). A more direct evidence that development of lymphedema is driven by repeated exposure to infective larvae and not so much by adult worms has been experimentally demonstrated in a primate model with B.malayi infections (2). This linear model of natural history of lymphatic filariasis has a vital bearing on the on-going global initiative for control of the disease. The model implies that infected subjects are prone for re-infection and effective blocking of transmission in human communities will be a critical requirement for successful control programme in disease endemic countries.

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