Parasite Immunology

Parasite Immunology, 2014, **36,** 338–346 DOI: 10.1111/pim.12081

Review Article

Immunology of lymphatic filariasis

S. BABU[1] & T. B. NUTMAN[2]

[1]NIAID-NIRT-ICER, Chennai, India, [2]Helminth Immunology Section, Laboratory of Parasitic Diseases, National Institute of Allergy and Infectious Diseases, Bethesda, MD, USA

SUMMARY

The immune responses to filarial parasites encompass a complex network of innate and adaptive cells whose interaction with the parasite underlies a spectrum of clinical manifestations. The predominant immunological feature of lymphatic filariasis is an antigen-specific Th2 response and an expansion of IL-10 producing $CD4^+$ T cells that is accompanied by a muted Th1 response. This antigen-specific T-cell hyporesponsiveness appears to be crucial for the maintenance of the sustained, long-standing infection often with high parasite densities. While the correlates of protective immunity to lymphatic filariasis are still incompletely understood, primarily due to the lack of suitable animal models to study susceptibility, it is clear that T cells and to a certain extent B cells are required for protective immunity. Host immune responses, especially $CD4^+$ T-cell responses clearly play a role in mediating pathological manifestations of LF, including lymphedema, hydrocele and elephantiasis. The main underlying defect in the development of clinical pathology appears to be a failure to induce T-cell hyporesponsiveness in the face of antigenic stimulation. Finally, another intriguing feature of filarial infections is their propensity to induce bystander effects on a variety of immune responses, including responses to vaccinations, allergens and to other infectious agents. The complexity of the immune response to filarial infection therefore provides an important gateway to understanding the regulation of immune responses to chronic infections, in general.

Keywords *B cells, cytokines, filariasis, helminths, parasites, T cells*

Correspondence: Subash Babu, National Institute for Research in Tuberculosis, No 1, Mayor Sathiyamurthy Road, Chetpet, Chennai – 600031, India (e-mail: sbabu@mail.nih.gov).
Received: 18 June 2013
Accepted for publication: 11 October 2013

INTRODUCTION

Lymphatic filariasis is an infection caused by three closely related nematode worms – *Wuchereria bancrofti, Brugia malayi* and *Brugia timori*. The parasites are transmitted by mosquitoes, and their life cycles in humans consist of adult worms living in the afferent lymphatic vessels while their progeny, the microfilariae, circulate in the peripheral blood. Although typically clinically asymptomatic, lymphatic filariasis can also be associated with lymphatic disease, which is responsible for considerable suffering, deformity and disability and is the second leading parasitic cause of disability with DALYs (disability-adjusted life years) estimated to be 5·549 million (1). Bancroftian filariasis, caused by *W. bancrofti,* is responsible for 90% of those with lymphatic filariasis (2) and the rest are caused by Brugian parasites. Lymphatic filariasis is a global health problem. At the present time (2013), the World Health Organization estimated that over 1·25 billion people are at risk in 72 countries and territories. It is known that approximately 120 million people are infected with filarial parasites and over 40 million have pathologic consequences, including the disfiguring elephantiasis. Clinical disease is manifested primarily as acute adenolymphangitis and chronic lymphedema that may lead to elephantiasis in men and women and to the formation of hydroceles (for *W. bancrofti* at least) in men.

All human filarial nematodes have a complex life cycle involving an insect vector, with Wuchereria and Brugia being transmitted by mosquitoes. Infection begins with the deposition of infective larvae (L3) in the skin during a mosquito bite. The larvae then enter through the puncture wound, reaching the lymphatics and migrate towards the lymph nodes. They reside within the lymphatics and lymph nodes and undergo a process of moulting and development to form L4 larvae and then adult worms. Following mating, the adult female releases live progeny called microfilariae that circulate in the bloodstream. These microfilariae can then be ingested by a mosquito during a

Published 2013. This article is a U.S. Government work and is in the public domain in the USA.

subsequent blood meal, where in they undergo development to form L2 and finally L3 larvae. The complex life cycle engenders a complicated host immune response, and it is this complexity of the host–parasite interaction that is thought to underlie the varied clinical manifestations of lymphatic filariasis. Lymphatic filariasis can manifest itself in a variety of clinical and subclinical conditions (2). The most common clinical manifestation is a relatively asymptomatic or subclinical infection characterized by the presence of circulating microfilariae and the relative absence of clinical symptoms. A subgroup of individuals present clinically with overt pathology in the form of lymphedema, hydrocele and elephantiasis. In this review, we discuss mostly studies pertaining to human filarial infections and have used the Brugia infection of mice as the primary model for studying host immune interaction with filarial parasites and referred to the Litomosoides model only in the absence of data from the Brugian model.

PROTOTYPICAL IMMUNE RESPONSES

The canonical host immune response to filarial parasites in both mice and humans is of the T helper 2 (Th2) type and involves the production of cytokines – IL-4, IL-5, IL-9, IL-10 and IL-13, the antibody isotypes – IgG1, IgG4 (in humans) and IgE, and expanded populations of eosinophils and alternatively activated macrophages (3). The initial interaction of T cells with a variety of host cell types including dendritic cells and macrophages induces and culminates in Th2 responses (3). While dendritic cells, basophils and innate lymphoid cells (ILCs) have been all shown to initiate Th2 responses in other helminth infections, their role in the initiation of early Th2 responses in filarial infections is still unclear. Type 2 ILCs have been shown to be important players in the initiation of Th2 responses in helminth infections (4, 5). Recent data from our laboratory have shown that ILC2 are expanded in both mouse models and human filarial infections and produce copious amount of IL-5 and IL-13 at times preceding the onset of classical Th2 responses (A. Boyd and T.B. Nutman, unpublished data). Thus, ILC2 might be important players in orchestrating the establishment of the Th2 response in filarial infections. These prototypical Th2 responses are modulated by both adaptive and natural regulatory T cells, alternatively activated macrophages, eosinophils and other cell populations over the duration of infection (6). The main characteristic of a chronic filarial infection appears to be the presence of a modified Th2 response and an IL-10 dominated regulatory environment (6).

T cells are the key players in immunity to filarial infections. Both nude mice (that lack T cells) (7, 8) and SCID or Rag-deficient mice (that lack both T and B cells) (9, 10) are susceptible to infection with Brugian parasites, indicating that T cells are absolutely critical for elimination of infection. It appears, however, that either T-cell subset – $CD4^+$ or $CD8^+$ T cells – can mediate resistance in nonpermissive animals because mice that lack either $CD4^+$ T cells or $CD8^+$ T cells are fully capable of resisting infection (11, 12). In addition, protective immunity to filarial infections in mice is dependent primarily on Th2 responses in mice. Thus, mice lacking IL-4, IL-4R or Stat6 (all deficient in Th2 responses) are all susceptible to infection with *Brugia* parasites (13, 14). Interestingly, IFNγ also appears to play an important role in protection against infection because mice lacking IFNγ exhibit impairment in the elimination of the parasite (13). Therefore, protective immunity to filarial infections requires coordination of both Th1 and Th2 responses (Figure 1).

One of the most consistent findings in filarial infections is the elevated level of IgE that is observed following L3 exposure (15). Most of the IgE produced is polyclonal IgE indicating a nonantigen-specific induction of IgE-producing B cells. (16). Indeed, these IgE antibodies remain detectable many years after the infection has been treated indicating the presence of long-lived memory B cells or plasma cells in filarial infections (17). IgE production both in mice and humans is absolutely dependent on IL-4 or IL-13. Other isotypes that are commonly elevated in chronically filarial-infected humans are IgG4 and IgG1, the former being most dependent on both IL-4 and IL-10 (18). The role of B cells in resistance to infection is less clear, although B cells, especially a specialized subset of B cells called B1 B cells, also appear to exert a major role in resistance to infection (19). Antibodies do play a major role in mediating protection to filarial infections. Thus, *in vivo* data from mice deficient in IgE showed increased worm burdens with *B. malayi* indicating an important role for IgE in host defence (20). Again using knockout mice models, IgM has also been shown to be crucial for host protection against *B. malayi* (21). In the lymphatics and lymph nodes as well as in the circulation, filarial parasites are susceptible to attack by the full range of host innate effector cells, including macrophages, eosinophils and neutrophils. The ability of these cells to kill the parasites is often dependent on one or more isotypes of specific antibody (often IgE but also IgM) and complement. Activated macrophages or granulocytes can release nitric oxide, damaging nitrogen intermediates and reactive oxygen species onto the surface of the parasites, but *in vivo* killing methods are not yet fully understood.

Dendritic cells are professional antigen-presenting cells (APCs) that play an essential role in presenting antigen to T cells to initiate immune responses, yet their role in filarial infections is not fully understood. It has been shown

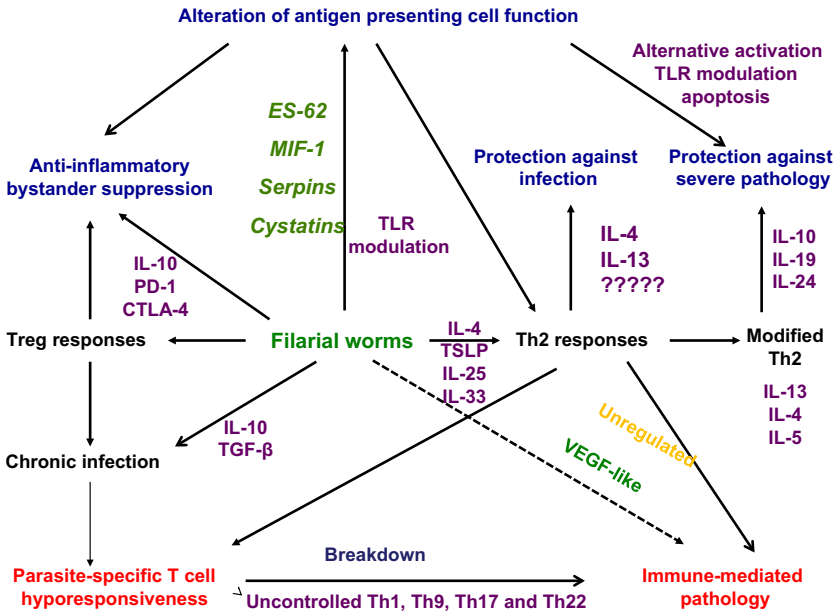

Figure 1 Regulation of the immune responses in filarial infections. The complex outcome of the interaction between the filarial parasite and the host immune system determines the immunological outcomes including (a) protection against infection; (b) parasite-specific T-cell hyporesponsiveness and alteration of APC function; (c) chronic infection; (d) protection against pathology and (e) anti-inflammatory bystander suppression.

that differentiation and maturation of DC in the presence of filarial antigens *in vitro* can stimulate Th2 responses with downmodulation of IL-12 production (22). In addition, live parasites have also been shown to induce cell death in human dendritic cells and diminish their capacity to activate $CD4^+$ T cells (22). Finally, asymptomatic filarial infection is characterized by increased numbers of circulating myeloid dendritic cells (defined as $Lineage^-$, $HLA\text{-}DR^+$, $CD11c^+$ cells) (23). On the other hand, human Langerhans cells [langerin(+) E-cadherin(+) CD1a(+)] exhibit minimal alterations in the cell surface activation markers or in mRNA expression of inflammation-associated genes, indicating a quiescent initial interaction of the parasite with human epidermal LC (24).

Macrophages are the other important class of antigen-presenting cells that can serve as protective effector cells in bacterial and protozoan infections by their production of nitric oxide and other mediators. A special class of macrophages is known to be induced in filarial infections, characterized by their preferential expression of the enzyme arginase, instead of nitric oxide due to increased activation of *arginase-1* by IL-4 and IL-13 (25). These macrophages, termed alternatively activated macrophages, have a very specific gene expression profile, with the ability to upregulate markers including *arginase-1*, chitinase 3-like proteins 3 and 4 (also known as *YM1* and *YM2*, respectively) and resistin-like molecule-α (RELMα) (26). These alternatively activated macrophages are known to be important in wound healing and are thought to help limit tissue immunopathology (27). By virtue of expression of regulatory molecules such as IL-10, TGFβ and programmed cell death 1 ligand 2 (PDL2), these macrophages might play a predominantly regulatory role in filarial infections (27). Interestingly, these filarial-induced macrophages appear to have the ability to expand locally and are less dependent on influx of monocytes from the bloodstream to perform their functions (28). While filarial infection does induce expression of these cells in humans, early interaction of parasites or parasite antigens leads to a predominantly pro-inflammatory response with expression of mainly pro-inflammatory cytokines including TNFα, IL-6 and IL-1β, as well as genes involved in inflammation and adhesion (29). Studies from murine models of filarial infection and *in vitro* data indicate that nitric oxide production by macrophages might be a key lethal hit in the host defence against the parasite (30). Therefore, the induction of alternatively activated macrophages might be an important immune evasion strategy for the parasites. Indeed, monocytes from patients with asymptomatic filarial infection also exhibit hallmarks of alternative activation, with diminished expression of inducible nitric oxide and enhanced expression of *arginase-1*, along with increased expression of resistin, mannose receptor C type 1 (MRC-1), macrophage galactose type C lectin (MGL) and chemokine ligand 18 (CCL18). Interestingly, live filarial parasites induce a monocyte phenotype that partly resem-

bles the alternatively activated state seen in infected individuals (31). Finally, monocytes, especially classical monocytes, appear to be remarkably efficient in antigen uptake in filarial infections (32).

Blood eosinophilia is characteristic of filarial infection and is mediated by IL-5 (probably in concert with IL-3 and GM-CSF) (33). Eosinophils are often the first cell type recruited to the site of infection, and eosinophilia occurs characteristically early following infection (33). Apart from the rapid kinetics of recruitment, eosinophils also exhibit morphological and functional changes attributable to eosinophil activation. These include changes in cell density, increased surface expression of activation markers, enhanced cellular cytotoxicity and release of granular proteins, cytokines, leukotrienes and other mediators of inflammation (33). Basophils have gained prominence recently due the possible role in Th2 cell differentiation as providers of initial IL-4 and even as APCs. Basophils in humans and mice readily generate large quantities of IL-4, in response to various stimuli, including filarial antigens, with or without dependence on IgE (34). In murine filarial infections, effector mechanisms involve multiple innate immune cells, with antibodies acting as initiators of immunity by activating Fc receptor-expressing cells. Basophils, by their ability to produce high levels of IL-4, act as effectors to promote filarial killing in secondary or challenge infections (35). Although eosinophils are crucial contributors to the early IL-4 pool, they are also important in protection against primary filarial infections (36, 37). The mechanism of protection mediated by eosinophils is thought to antibody-dependent cell-mediated cytotoxicity or through release of eosinophil granule contents.

IMMUNE EVASION

Filarial parasites exert profound immunoregulatory effects on the host immune system with both parasite antigen specific and more generalized levels of immune modulation. The main mechanisms of immune evasion include immunosuppression, immunological tolerance and modification of stereotypical Th2 responses (3). Immunosuppression and immunological tolerance are characterized typically by immunoregulatory cytokine-induced suppression of immune responses and a state of immune tolerance in effector T cells, respectively. On the other hand, in the modified Th2 response, antibody isotype switching to the noninflammatory isotype IgG4 (in humans) and induction of alternatively activated macrophages occurs (3). It has been shown that patients with lymphatic filariasis have markedly diminished responses to parasite antigens and in addition some nonspecific inhibition of responses to bystander antigens (38). Thus, while host immunosuppression is usually antigen specific, chronic infection is often associated with spillover effects on third party antigens as well.

Among the notable immune evasion strategies, a key one is the secretion of products that modulate host immune function. Phosphorylcholine (PC) is a small hapten-like moiety present in the excretory/secretory products of many helminths, and one particular PC containing molecule called ES-62 from filarial worms has been shown to have a wide variety of immunomodulatory properties (39). Thus, ES-62 can downregulate the proliferation of $CD4^+$ T cells and conventional B cells, decrease IL-4 and IFNγ production, upregulate proliferation and IL-10 production by B1 B cells and condition APCs to drive Th2 differentiation with concomitant inhibition of Th1 responses (40). Similarly, the presence of cytokine- and chemokine-like molecules in filarial parasites has been shown to have functional effects on the host innate immune responses as is the case with TGFβ and macrophage migration inhibitory factors (MIF) homologs (41). In addition, a recent analysis of the filarial parasite genome has identified a remarkable number of human cytokine and chemokine mimics and/or antagonists within the parasite genome. These include members of the interleukin-16 (IL-16) family, an IL-5 receptor antagonist, an interferon regulatory factor, a homolog of suppressor of cytokine signalling 7 (SOCS7) and two members of the chemokine-like family. Moreover, other potential immunomodulators have been demonstrated to be present in the filarial genome, including serpins and cystatins (modulation of antigen processing and presentation to T cells), indoleamine 2,3-dioxygenase (IDO) genes (potent immunomodulatory molecule), and Wnt family of developmental regulators (modulation of immune activation) (42).

The induction of regulatory T cells, modulation of effector T cell and APCs and apoptosis of responder cells (43) have been suggested to be the major factors influencing the regulation of the immune response in filarial infection. Regulatory T cells (both natural and adaptive) have been postulated to play a role in the immune evasion mechanism of the parasite. IL-10 and TGFβ, both factors associated with regulatory T cells, are elicited in response to helminth infections, and *in vitro* neutralization of IL-10 and TGFβ has been demonstrated to partially restore both T-cell proliferation and cytokine production in lymphatic filariasis (44). Similarly, evidence from mouse models argues for a major role of natural Tregs ($CD25^+Foxp3^+$ Treg cells) in immunity during filarial infections (45–47). In murine filarial infections, parasite survival is directly linked to Treg activity and immunity to infection can be restored by elimination of Tregs (45). Effector T-cell responses can be turned off or modulated through a

variety of mechanisms including through CTLA-4 and PD-1. Interestingly, increased expression of CTLA-4 and PD-1 has been demonstrated in human filarial infections, and blocking of CTLA-4 can restore partially a degree of immunological responsiveness in cells from infected individuals (48, 49). Similar findings have also been observed in murine models of filarial infection (50). Finally, classical signs of anergy have been demonstrated in T cells of filarial-infected individuals with decreased IL-2 production and increased expression of E3 ubiquitin ligases (49).

Filarial parasites induce downmodulation of MHC class I and class II as well as cytokines and other genes involved in antigen presentation in dendritic cells, thereby rendering them less capable of activating $CD4^+$ T cells (22). In addition, live parasites have also been shown to cause downregulation of MHC class II and IL-18 and multiple genes involved in antigen presentation in skin-resident LC (51). Filarial parasite interaction with macrophages has been shown to induce alternatively activated macrophages. By virtue of expression of expressing regulatory molecules such as IL-10, TGFβ and PDL2, these macrophages may also have a predominantly regulatory role in filarial infections (52). These anti-inflammatory macrophages can suppress T-cell responses through arginase-1 production and PDL2 expression and inhibit classical macrophage inflammation and recruitment through arginase-1, RELMα, triggering receptor expressed on myeloid cells 2 (TREM2) and other molecules (53). Another mechanism of immune evasion is the ability of filarial parasites to induce host cell apoptosis (54). Apoptosis of $CD4^+$ T cells has been demonstrated *in vivo* in experimental models of filarial infection in mice. In addition, *Brugia* microfilariae have been shown to interact with dendritic cells and NK cells and subsequently induce their apoptosis (22, 55).

One of the main characteristics of asymptomatic or subclinical filarial infection is the modulation of TLR expression and function in a variety of cell types including B cells, T cells and monocytes (56, 57). Either homeostatic or antigen-stimulated expression of certain TLRs was shown to be diminished in B cells, T cells and monocytes of filarial-infected individuals. In addition, TLR stimulation of both APCs and T cells leads to diminished activation/cytokine production, indicating a state of immune regulation. Furthermore, live filarial parasites have the capacity to downregulate TLR expression (specifically TLR3 and 4) on dendritic cells as well (58). This is accompanied by an impaired ability of dendritic cells to produce certain cytokines in response to TLR3 and 4 ligands. The diminished expression and function of TLR on immune cells is thought to be a likely consequence of chronic antigen stimulation and probably serves as a novel mechanism to protect against the development of pathology in filarial disease (59).

While most of the immunological studies in filarial infections have focused on filarial antigen-induced immune responses, the study of the immune responses engendered by live parasites provides some interesting details (49). Live parasites cause a significant impairment of both Th1 and Th2 cytokines in response to both L3 and Mf stages with diminished production of Th1 (IFNγ and TNF-α) and Th2 (IL-4 and IL-5) cytokines. This is accompanied by an impaired induction of T-bet (the master Th1 transcription factor) and GATA-3 (the master Th2 transcription factor) mRNA and by significantly increased expression of Foxp3, TGFβ, CTLA-4, PD-1, ICOS and IDO. In addition, the compromise of effector T-cell function is mediated by the enhanced induction of anergy-inducing factors – cbl-b, c-cbl, Itch and Nedd4. Finally, blocking CTLA-4 or neutralizing TGFβ restored the ability to mount Th1/Th2 responses and reversed the induction of anergy-inducing factors. Thus, a variety of regulatory factors including IL-10, TGFβ, nTregs (perhaps via PD-1 and CTLA-4) have been implicated in the downmodulation of immune responses in patent filarial infection and might have a potentially vital role in the establishment of chronic, asymptomatic infection.

PATHOGENESIS OF FILARIAL DISEASE

The most severe clinical manifestations of lymphatic filariasis are lymphedema and elephantiasis. The first major insights into the role of lymphatic damage in the pathogenesis of lymphatic filarial disease came from studies using Brugian infections of animals. Infection of normal or nude (lacking T cells) mice resulted in lymphangitis and peri-lymphangitis in both groups of mice with acute and chronic inflammation predominating in the former (60). Interestingly, as normal mice are not permissive to infection, lymphangiectasia was observed to progress only in nude mice. While infection of nude mice was characterized predominantly by lymphangiectasia, reconstitution of these mice with spleen cells from normal mice (thereby restoring normal adaptive immunity) resulted in progressive fibrosis, obliterative lymph thrombus formation, interstitial infiltrates and extensive perilympangitis (61). Similarly, studies using SCID mice (lacking both T and B cells) showed that lymphangitis and lymphangiectasia were classical features of infection in the absence of adaptive immunity and that reconstitution with spleen cells from normal mice resulted in progressive disease (9).

Pro-inflammatory cytokines of innate origin also appear to play an important role in Brugian infection because infection of nude mice results in elevated levels of IL-1,

IL-6, TNF-α and GM-CSF in lymph fluid (62). Therefore, innate cytokines appear to play a prominent role in the initiation of pathology in filarial-infected animal models. The importance of pro-inflammatory cytokines, possibly of innate origin, in the pathogenesis of lymphedema, has been further strengthened by a series of studies in humans in either the early or late stages or lymphedema. Studies have shown that individuals with chronic lymphatic pathology have elevated levels of C-reactive protein (an acute phase protein, indicating an acute inflammatory response), pro-inflammatory cytokines such as TNF-α, IL-6 and soluble TNF receptor, endothelin-1, IL-2, as well as IL-8, MIP-1α, MIP-1β, MCP-1, TARC and IP-10 in the peripheral circulation (6). Similarly, while patients with both acute and chronic manifestations of LF have elevated circulating levels of IL-6 and IL-8, only those with chronic disease manifestations have elevated levels of sTNF receptors (63). Another important mechanism of immune activation in chronic infections is the occurrence of microbial translocation with elevations in the circulating levels of microbial products. Microbial translocation across the intestine or across the lymphatics could possibly contribute to inflammation and innate immune activation. Indeed, we have shown that increased circulating levels of LPS (which serves as a marker for microbial translocation) and decreased levels of LPS-binding protein (LBP) are characteristic features of filarial lymphatic pathology (64). We have also demonstrated that this process is associated with development of an acute phase response and the presence of markers of inflammation in plasma – CRP, alpha-2 macroglobulin, serum amyloid protein-A and haptoglobin (64). Moreover, increased serum levels of proinflammatory cytokines – IL-1β, IL-12, TNF-α and IL-6, are associated with progressive immune activation in filarial pathology. As filarial lymphedema is known to be associated with increased bacterial and fungal loads in the lymphatics, we postulate that microbial translocation across the damaged lymphatics in filarial lymphedema is a novel source of immune activation.

Apart from systemic immune activation, progressive fibrosis and extracellular matrix remodelling is another salient feature of filarial pathology. Matrix metalloproteinases (MMPs) are proteolytic enzymes that control matrix remodelling and collagen turnover. These MMPs and their inhibitors [tissue inhibitors of metalloproteinases (TIMPs)] are produced by a variety of cell types including macrophages, granulocytes, epidermal cells and fibroblasts. The dysregulation of MMPs and TIMPs is known to underlie the development of pathology in several infections, including viral, bacterial, spirochetal, protozoan, fungal and parasitic infections. Along the same lines, recent data suggest that an increase in circulating levels of MMPs and TIMPs is characteristic of the filarial disease process and that that altered ratios of MMP/TIMP are an important underlying factor in the pathogenesis of tissue fibrosis in filarial lymphatic disease. In addition, this is correlated with elevated levels of Type 2 cytokines known to be intimately involved in fibrosis – IL-5, IL-13 and TGFβ (65). Another study has also examined the alterations in pro-fibrotic factors in filarial pathology and revealed that increased levels of basic fibroblast growth factor (bFGF) and placental growth factor (PIGF) can also occur in filarial lymphedema patients (66). Thus, filarial pathology arises out of a complex early interplay between the parasite and the hosts innate responses and its tissue homeostasis.

Studies in animals also implicate an important role for endothelial cells in pathogenesis of lymphatic dysfunction because these cells exhibit morphological alterations upon chronic infection. Indeed, live filarial parasites (and their excretory/secretory products) induce activation, proliferation and tube formation in lymphatic endothelial cells (LECs) (67). Moreover, only serum from patently infected or diseased individuals was shown to induce significant LEC proliferation. This lymphatic remodelling recapitulates the observations seen *in vivo* in immunodeficient mice. Vascular endothelial growth factor (VEGF) family members have also been implicated in lymphangiogenesis. It was recently shown that lymphatic endothelial-specific VEGF-C levels are significantly elevated in individuals with filarial disease (68). The other VEGF family member that has been implicated to play a role in filarial disease is VEGF-A (69). Elevated levels of VEGF-A and endothelin-1 have been observed in the serum of filarial-infected individuals, and more specifically, VEGF-A has been implicated to play a role in the development of hydrocele due to its ability to induce increased vascular density, enhance leucocyte adhesion and promote lymphangiogenesis (70). Thus, excess secretion of pro-inflammatory cytokines and angiogenic factors like VEGF-A could result in extravasation and accumulation of fluids, plasma and lymph from the blood and lymphatic vessels into the scrotum resulting in the formation of hydrocele. Other angiogenic factors such as angiopoietins-1 and 2 are also found at elevated levels in individuals with filarial-induced pathology (66).

In terms of cellular subsets, it was first discovered that individuals with chronic lymphedema have increased frequencies of activated $CD8^+$ T cells (expressing HLA-DR) in the peripheral blood (71). Later, it was also shown that the frequency of $CD8^+$ T cells in tissues (including skin and subcutaneous tissues) was increased as well (72). Indeed, biopsy specimens from affected tissues exhibited increased levels of VCAM-1, and PBMC supernatants from diseased individuals showed the capacity to upregu-

late both MHC class I molecules and VCAM-1 on endothelial cell cultures (73, 74). Moreover, TCR Vβ phenotyping revealed a biased TCR repertoire in the T cells infiltrating the affected tissues in diseased individuals (75). In addition, examination of chemokine receptor expression on T, B and NK cells revealed a significant increase in the frequencies of circulating CCR9 – expressing T and B cells (76). Because CCR9 is not normally expressed on circulating T and B cells, these results suggest that chemokine receptors (particularly CCR9) are involved in the pathogenesis of lymphatic filarial disease and that trafficking of particular cellular subsets may influence clinical outcome. Unpublished data utilizing multiparameter flow cytometry have failed to reveal any significant difference in the frequencies of circulating naïve, effector memory and central memory $CD4^+$ and $CD8^+$ T cells in patients with filarial disease compared to those with long-standing infection. Thus, alterations in T-cell numbers and function, especially at the site of pathology, are probably of major importance in pathogenesis.

Using multicolour flow cytometry, we have been able to show that the frequency of Th1 cells ($CD4^+$ T cells expressing either IFNγ or IL-2 or TNF-α); Th9 cells ($CD4^+$ T cells expressing IL-9 and IL-10); Th17 cells ($CD4^+$ T cells expressing IL-17) and Th22 cells ($CD4^+$ T cells expressing IL-22) is significantly enhanced in filarial pathology. This is accompanied by a concomitant decrease in the frequency of Th2 cells ($CD4^+$ T cells expressing IL-4 or IL-5 or IL-13) both at homeostasis and following parasite antigen stimulation (data not published). Although less well studied than Th1 cells, Th17 cells might also have an important role in the pathogenesis of disease in filarial infection because PBMC from individuals with pathology (but not asymptomatic patients) express significantly higher levels of the Th17-associated cytokines as well as the master transcription factor – RORC at the mRNA level (77). Finally, pathology in lymphatic filariasis is also associated with expanded frequencies of Th9 cells, $CD4^+$ T cells that express both IL-9 and IL-10, but not IL-4, and this frequency exhibits a positive correlation with the severity of lymphedema in filarial infections (78). Therefore, immunopathology in lymphatic filariasis appears to be mostly associated with poor regulation of effector $CD4^+$ and $CD8^+$ T cells that can unleash pro-inflammatory Th1, Th9 and Th17 type immune responses. How, these pro-inflammatory Th1, Th9 and Th17 cells interact with innate cells, endothelial cells and other target cells to initiate and propagate lymphatic damage, and tissue fibrosis remains to be elucidated.

CONCLUSIONS

Filarial infections are a classic example of chronic infections in which complete elimination of all parasites is rarely achieved, presumably because sterilizing immunity might necessitate deleterious host immune responses. Therefore, immune-mediated pathology is often associated with disease manifestations in these infections. The optimal host response is one which balances parasite control at the levels in which the parasite load can be tolerated and maintenance of immune homeostasis without irreparable tissue damage. Filarial infections, for the majority of those infected, reflect immune system-parasite balance, a balance that, when it fails, results in significant immune-mediated inflammation and pathology. Interestingly, this is also reflected in the disease manifestations of the related filarial parasite, Onchocerca volvulus, where chronic, asymptomatic infection is associated with loss of parasite control and the hyper-reactive, pro-inflammatory state is associated with severe chronic papular dermatitis and hyperpigmentation (sowda) (79).

ACKNOWLEDGEMENTS

This work was supported by the Intramural Research Program of the Division of Intramural Research, National Institute of Allergy and Infectious Diseases, National Institutes of Health.

DISCLOSURE

Because S. Babu and T. B. Nutman are government employees and this is a government work, the work is in the public domain in the United States. Notwithstanding any other agreements, the NIH reserves the right to provide the work to PubMedCentral for display and use by the public, and PubMedCentral may tag or modify the work consistent with its customary practices. You can establish rights outside of the U.S. subject to a government use licence.

REFERENCES

1 Fenwick A. The global burden of neglected tropical diseases. *Public Health* 2012; **126**: 233–236.
2 Lymphatic filariasis: the disease and its control. Fifth report of the WHO Expert Committee on Filariasis. *World Health Organ Tech Rep Ser* 1992; **821**: 1–71.
3 Allen JE & Maizels RM. Diversity and dialogue in immunity to helminths. *Nat Rev Immunol* 2011; **11**: 375–388.
4 Neill DR, Wong SH, Bellosi A, *et al.* Nuocytes represent a new innate effector leukocyte that mediates type-2 immunity. *Nature* 2010; **464**: 1367–1370.

5 Licona-Limon P, Kim LK, Palm NW & Flavell RA. TH2, allergy and group 2 innate lymphoid cells. *Nat Immunol* 2013; **14**: 536–542.

6 Babu S & Nutman TB. Immunopathogenesis of lymphatic filarial disease. *Semin Immunopathol* 2012; **34**: 847–861.

7 Suswillo RR, Owen DG & Denham DA. Infections of Brugia pahangi in conventional and nude (athymic) mice. *Acta Trop* 1980; **37**: 327–335.

8 Vincent AL, Sodeman WA & Winters A. Development of Brugia pahangi in normal and nude mice. *J Parasitol* 1980; **66**: 448.

9 Nelson FK, Greiner DL, Shultz LD & Rajan TV. The immunodeficient scid mouse as a model for human lymphatic filariasis. *J Exp Med* 1991; **173**: 659–663.

10 Babu S, Shultz LD, Klei TR & Rajan TV. Immunity in experimental murine filariasis: roles of T and B cells revisited. *Infect Immun* 1999; **67**: 3166–3167.

11 Rajan TV, Nelson FK, Shultz LD, Koller BH & Greiner DL. $CD8^+$ T lymphocytes are not required for murine resistance to human filarial parasites. *J Parasitol* 1992; **78**: 744–746.

12 Rajan TV, Greiner DL, Yates JA & Shultz LD. Growth of the human filarial parasite Brugia malayi in mice lacking major histocompatibility complex class II antigen expression. *Acta Trop* 1996; **61**: 267–271.

13 Babu S, Ganley LM, Klei TR, Shultz LD & Rajan TV. Role of gamma interferon and interleukin-4 in host defense against the human filarial parasite Brugia malayi. *Infect Immun* 2000; **68**: 3034–3035.

14 Spencer L, Shultz L & Rajan TV. Interleukin-4 receptor-Stat6 signaling in murine infections with a tissue-dwelling nematode parasite. *Infect Immun* 2001; **69**: 7743–7752.

15 Hussain R, Hamilton RG, Kumaraswami V, Adkinson NF Jr & Ottesen EA. IgE responses in human filariasis. I. Quantitation of filaria-specific IgE. *J Immunol* 1981; **127**: 1623–1629.

16 Hussain R & Ottesen EA. IgE responses in human filariasis. III. Specificities of IgE and IgG antibodies compared by immunoblot analysis. *J Immunol* 1985; **135**: 1415–1420.

17 Mitre E & Nutman TB. IgE memory: persistence of antigen-specific IgE responses years after treatment of human filarial infections. *J Allergy Clin Immunol* 2006; **117**: 939–945.

18 Nutman TB & Kumaraswami V. Regulation of the immune response in lymphatic filariasis: perspectives on acute and chronic infection with *Wuchereria bancrofti* in South India. *Parasite Immunol* 2001; **23**: 389–399.

19 Paciorkowski N, Porte P, Shultz LD & Rajan TV. B1 B lymphocytes play a critical role in host protection against lymphatic filarial parasites. *J Exp Med* 2000; **191**: 731–736.

20 Spencer LA, Porte P, Zetoff C & Rajan TV. Mice genetically deficient in immunoglobulin E are more permissive hosts than wild-type mice to a primary, but not secondary, infection with the filarial nematode Brugia malayi. *Infect Immun* 2003; **71**: 2462–2467.

21 Rajan B, Ramalingam T & Rajan TV. Critical role for IgM in host protection in experimental filarial infection. *J Immunol* 2005; **175**: 1827–1833.

22 Semnani RT, Liu AY, Sabzevari H, *et al.* Brugia malayi microfilariae induce cell death in human dendritic cells, inhibit their ability to make IL-12 and IL-10, and reduce their capacity to activate $CD4^+$ T cells. *J Immunol* 2003; **171**: 1950–1960.

23 Semnani RT, Mahapatra L, Dembele B, *et al.* Expanded numbers of circulating myeloid dendritic cells in patent human filarial infection reflect lower CCR1 expression. *J Immunol* 2010; **185**: 6364–6372.

24 Boyd A, Bennuru S, Wang Y, *et al.* Quiescent innate response to infective filariae by human langerhans cells suggests a strategy of immune evasion. *Infect Immun* 2013; **81**: 1420–1429.

25 Loke P, MacDonald AS, Robb A, Maizels RM & Allen JE. Alternatively activated macrophages induced by nematode infection inhibit proliferation via cell-to-cell contact. *Eur J Immunol* 2000; **30**: 2669–2678.

26 Nair MG, Gallagher IJ, Taylor MD, *et al.* Chitinase and Fizz family members are a generalized feature of nematode infection with selective upregulation of Ym1 and Fizz1 by antigen-presenting cells. *Infect Immun* 2005; **73**: 385–394.

27 Allen JE & Wynn TA. Evolution of Th2 immunity: a rapid repair response to tissue destructive pathogens. *PLoS Pathog* 2011; **7**: e1002003.

28 Jenkins SJ, Ruckerl D, Cook PC, *et al.* Local macrophage proliferation, rather than recruitment from the blood, is a signature of TH2 inflammation. *Science* 2011; **332**: 1284–1288.

29 Chaussabel D, Semnani RT, McDowell MA, Sacks D, Sher A & Nutman TB. Unique gene expression profiles of human macrophages and dendritic cells to phylogenetically distinct parasites. *Blood* 2003; **102**: 672–681.

30 Rajan TV, Porte P, Yates JA, Keefer L & Shultz LD. Role of nitric oxide in host defense against an extracellular, metazoan parasite, Brugia malayi. *Infect Immun* 1996; **64**: 3351–3353.

31 Babu S, Kumaraswami V & Nutman TB. Alternatively activated and immunoregulatory monocytes in human filarial infections. *J Infect Dis* 2009; **199**: 1827–1837.

32 Semnani RT, Keiser PB, Coulibaly YI, *et al.* Filaria-induced monocyte dysfunction and its reversal following treatment. *Infect Immun* 2006; **74**: 4409–4417.

33 Klion AD & Nutman TB. The role of eosinophils in host defense against helminth parasites. *J Allergy Clin Immunol* 2004; **113**: 30–37.

34 Mitre E, Taylor RT, Kubofcik J & Nutman TB. Parasite antigen-driven basophils are a major source of IL-4 in human filarial infections. *J Immunol* 2004; **172**: 2439–2445.

35 Torrero MN, Hubner MP, Larson D, Karasuyama H & Mitre E. Basophils amplify type 2 immune responses, but do not serve a protective role, during chronic infection of mice with the filarial nematode Litomosoides sigmodontis. *J Immunol* 2010; **185**: 7426–7434.

36 Ramalingam T, Porte P, Lee J & Rajan TV. Eosinophils, but not eosinophil peroxidase or major basic protein, are important for host protection in experimental Brugia pahangi infection. *Infect Immun* 2005; **73**: 8442–8443.

37 Simons JE, Rothenberg ME & Lawrence RA. Eotaxin-1-regulated eosinophils have a critical role in innate immunity against experimental Brugia malayi infection. *Eur J Immunol* 2005; **35**: 189–197.

38 Metenou S, Babu S & Nutman TB. Impact of filarial infections on coincident intracellular pathogens: *Mycobacterium tuberculosis* and *Plasmodium falciparum*. *Curr Opin HIV AIDS* 2012; **7**: 231–238.

39 Harnett W, McInnes IB & Harnett MM. ES-62, a filarial nematode-derived immunomodulator with anti-inflammatory potential. *Immunol Lett* 2004; **94**: 27–33.

40 Harnett W & Harnett MM. Helminth-derived immunomodulators: can understanding the worm produce the pill? *Nat Rev Immunol* 2010; **10**: 278–284.

41 Hewitson JP, Grainger JR & Maizels RM. Helminth immunoregulation: the role of parasite secreted proteins in modulating host immunity. *Mol Biochem Parasitol* 2009; **167**: 1–11.

42 Desjardins CA, Cerqueira GC, Goldberg JM, *et al.* Genomics of Loa loa, a Wolbachia-free filarial parasite of humans. *Nat Genet* 2013; **45**: 495–500.

43 Maizels RM & Yazdanbakhsh M. Immune regulation by helminth parasites: cellular and molecular mechanisms. *Nat Rev Immunol* 2003; **3**: 733–744.

44 King CL, Mahanty S, Kumaraswami V, *et al.* Cytokine control of parasite-specific anergy in human lymphatic filariasis. Preferential induction of a regulatory T helper type 2 lymphocyte subset. *J Clin Invest* 1993; **92**: 1667–1673.

45 Taylor MD, LeGoff L, Harris A, Malone E, Allen JE & Maizels RM. Removal of regulatory T cell activity reverses hyporesponsiveness and leads to filarial parasite clearance in vivo. *J Immunol* 2005; **174**: 4924–4933.

46 Gillan V & Devaney E. Regulatory T cells modulate Th2 responses induced by Brugia pahangi third-stage larvae. *Infect Immun* 2005; **73**: 4034–4042.

47 Taylor MD, van der Werf N, Harris A, *et al.* Early recruitment of natural $CD4^+$ $Foxp3^+$ Treg cells by infective larvae determines the outcome of filarial infection. *Eur J Immunol* 2009; **39**: 192–206.

48 Steel C & Nutman TB. CTLA-4 in filarial infections: implications for a role in diminished T cell reactivity. *J Immunol* 2003; **170**: 1930–1938.

49 Babu S, Blauvelt CP, Kumaraswami V & Nutman TB. Regulatory networks induced by live parasites impair both Th1 and Th2 pathways in patent lymphatic filariasis:

implications for parasite persistence. *J Immunol* 2006; **176**: 3248–3256.
50 van der Werf N, Redpath SA, Azuma M, Yagita H & Taylor MD. Th2 cell-intrinsic hypo-responsiveness determines susceptibility to helminth infection. *PLoS Pathog* 2013; **9**: e1003215.
51 Semnani RT, Law M, Kubofcik J & Nutman TB. Filaria-induced immune evasion: suppression by the infective stage of Brugia malayi at the earliest host-parasite interface. *J Immunol* 2004; **172**: 6229–6238.
52 Allen JE & Loke P. Divergent roles for macrophages in lymphatic filariasis. *Parasite Immunol* 2001; **23**: 345–352.
53 Loke P, Gallagher I, Nair MG, *et al.* Alternative activation is an innate response to injury that requires $CD4^+$ T cells to be sustained during chronic infection. *J Immunol* 2007; **179**: 3926–3936.
54 Jenson JS, OConnor R, Osborne J & Devaney E. Infection with Brugia microfilariae induces apoptosis of CD4(+) T lymphocytes: a mechanism of immune unresponsiveness in filariasis. *Eur J Immunol* 2002; **32**: 858–867.
55 Babu S, Blauvelt CP & Nutman TB. Filarial parasites induce NK cell activation, type 1 and type 2 cytokine secretion, and subsequent apoptotic cell death. *J Immunol* 2007; **179**: 2445–2456.
56 Babu S, Blauvelt CP, Kumaraswami V & Nutman TB. Diminished expression and function of TLR in lymphatic filariasis: a novel mechanism of immune dysregulation. *J Immunol* 2005; **175**: 1170–1176.
57 Babu S, Blauvelt CP, Kumaraswami V & Nutman TB. Cutting edge: diminished T cell TLR expression and function modulates the immune response in human filarial infection. *J Immunol* 2006; **176**: 3885–3889.
58 Semnani RT, Venugopal PG, Leifer CA, Mostbock S, Sabzevari H & Nutman TB. Inhibition of TLR3 and TLR4 function and expression in human dendritic cells by helminth parasites. *Blood* 2008; **112**: 1290–1298.
59 Venugopal PG, Nutman TB & Semnani RT. Activation and regulation of Toll-Like Receptors (TLRs) by helminth parasites. *Immunol Res* 2009; **43**: 252–263.
60 Vincent AL, Vickery AC, Lotz MJ & Desai U. The lymphatic pathology of Brugia pahangi in nude (athymic) and thymic mice C3H/HeN. *J Parasitol* 1984; **70**: 48–56.
61 Vickery AC, Albertine KH, Nayar JK & Kwa BH. Histopathology of Brugia malayi-infected nude mice after immune-reconstitution. *Acta Trop* 1991; **49**: 45–55.
62 Rao UR, Vickery AC, Kwa BH & Nayar JK. Regulatory cytokines in the lymphatic pathology of athymic mice infected with Brugia malayi. *Int J Parasitol* 1996; **26**: 561–565.
63 Satapathy AK, Sartono E, Sahoo PK, *et al.* Human bancroftian filariasis: immunological markers of morbidity and infection. *Microbes Infect* 2006; **8**: 2414–2423.
64 Anuradha R. Circulating microbial products and acute phase proteins as markers of pathogenesis in lymphatic filarial disease. *PLoS Pathog* 2012; **8**: e1002749.
65 Anuradha R. Altered circulating levels of matrix metalloproteinases and inhibitors associated with elevated Type 2 cytokines in lymphatic filarial disease. *PLoS Negl Trop Dis* 2012; **6**: e1681.
66 Bennuru S, Maldarelli G, Kumaraswami V, Klion AD & Nutman TB. Elevated levels of plasma angiogenic factors are associated with human lymphatic filarial infections. *Am J Trop Med Hyg* 2010; **83**: 884–890.
67 Bennuru S & Nutman TB. Lymphangiogenesis and lymphatic remodeling induced by filarial parasites: implications for pathogenesis. *PLoS Pathog* 2009; **5**: e1000688.
68 Debrah AY, Mand S, Specht S, *et al.* Doxycycline reduces plasma VEGF-C/sVEGFR-3 and improves pathology in lymphatic filariasis. *PLoS Pathog* 2006; **2**: e92.
69 Debrah AY, Mand S, Toliat MR, *et al.* Plasma vascular endothelial growth Factor-A (VEGF-A) and VEGF-A gene polymorphism are associated with hydrocele development in lymphatic filariasis. *Am J Trop Med Hyg* 2007; **77**: 601–608.
70 Debrah AY, Mand S, Marfo-Debrekyei Y, *et al.* Reduction in levels of plasma vascular endothelial growth factor-A and improvement in hydrocele patients by targeting endosymbiotic *Wolbachia* sp. in *Wuchereria bancrofti* with doxycycline. *Am J Trop Med Hyg* 2009; **80**: 956–963.
71 Lal RB, Kumaraswami V, Krishnan N, Nutman TB & Ottesen EA. Lymphocyte subpopulations in Bancroftian filariasis: activated (DR+) $CD8^+$ T cells in patients with chronic lymphatic obstruction. *Clin Exp Immunol* 1989; **77**: 77–82.
72 Freedman DO, Horn TD, Maia e Silva CM, Braga C & Maciel A. Predominant $CD8^+$ infiltrate in limb biopsies of individuals with filarial lymphedema and elephantiasis. *Am J Trop Med Hyg* 1995; **53**: 633–638.
73 Freedman DO, Nutman TB, Jamal S, Kumaraswami V & Ottesen EA. Selective up-regulation of endothelial cell class I MHC expression by cytokines from patients with lymphatic filariasis. *J Immunol* 1989; **142**: 653–658.
74 Freedman DO, Parker-Cook SMaia e Silva MC, Braga C & Maciel A. Very late antigen-4/vascular cell adhesion molecule-1 (VLA-4/VCAM-1) pathway is involved in the transendothelial migration of lymphocytes in bancroftian filariasis. *J Immunol* 1996; **156**: 2901–2908.
75 Freedman DO, Plier DA, de Almeida A, *et al.* Biased TCR repertoire in infiltrating lesional T cells in human Bancroftian filariasis. *J Immunol* 1999; **162**: 1756–1764.
76 Babu S, Blauvelt CP, Kumaraswami V & Nutman TB. Chemokine receptors of T cells and of B cells in lymphatic filarial infection: a role for CCR9 in pathogenesis. *J Infect Dis* 2005; **191**: 1018–1026.
77 Babu S, Bhat SQ, Pavan Kumar N, *et al.* Filarial lymphedema is characterized by antigen-specific Th1 and th17 proinflammatory responses and a lack of regulatory T cells. *PLoS Negl Trop Dis* 2009; **3**: e420.
78 Anuradha R, George PJ, Hanna LE, *et al.* IL-4-, TGF-β-, and IL-1-dependent expansion of parasite antigen-specific Th9 cells is associated with clinical pathology in human lymphatic filariasis. *J Immunol* 2013; **191**: 2466–2473.
79 Taylor MJ, Hoerauf A & Bockarie M. Lymphatic filariasis and onchocerciasis. *Lancet* 2010; **376**: 1175–1185.