Regulation of the immune response in lymphatic filariasis and onchocerciasis

Christopher L. King and Thomas B. Nutman

The persistence of microfilariae in the blood or skin accompanied by a prominent eosinophilia and elevated serum IgE levels are common features of human infection with filarial parasites. In this review Christopher King and Thomas Nutman discuss recent findings on the role of interleukin 4 (IL-4), IL-5 and gamma-interferon (IFN-γ) in the induction of these immediate hypersensitivity responses. They discuss the role of hypersensitivity in immunity, the development of immune tolerance to filarial antigens and suggest that could explain the impaired immune response of some individuals to filarial infections and the persistence of the microfilaremic state.

Among the eight filarial parasites that commonly infect humans, *Onchocerca volvulus* (the organism responsible for onchocerciasis, or river blindness) and *Wuchereria bancrofti* and *Brugia malayi* (the causative agents of lymphatic filariasis) account for most human filarial disease. For all filarial parasites, infection of the human host is initiated by the deposition of third stage larvae (L3) in the skin after a bite by an infected arthropod. The larvae mature and develop into subcutaneous or lymph-dwelling adult worms over a period of months to years. Mature female worms produce and subsequently release microfilariae into the bloodstream (lymphatic filariases) or into the skin (onchocerciasis) where they are available for uptake by the intermediate arthropod host and may initiate clinical symptoms.

The range of clinical manifestations of both onchocerciasis and lymphatic filariasis is broad, and the diversity of clinical responses to filarial infection is considered to reflect the intensity and type of immune response to the parasite or parasite products (Fig. 1)[1]. However, most individuals in filarial-endemic regions are asymptomatic and microfilaria-positive. This group is generally considered to be immunologically hyporesponsive to the parasite, as they have relatively low serum levels of antifilarial antibodies[2,3] and a poor ability to mount B- and T-cell responses to parasite antigens *in vitro*[4–6]. They are unable to clear their microfilariae, and serve as the reservoir for continued transmission of the parasite. In contrast, patients with pathology associated with infection (for example elephantiasis, filarial fevers and severe onchodermatitis) are characteristically microfilaria-negative but have relatively strong cellular and humoral responses to the parasite. It is this response that is thought to induce the pathology seen in these infections. A third group that has no evidence of infection despite high levels of exposure to the parasite has also been identified; these individuals are postulated to be 'putatively immune' and have the most marked T-cell responses to filarial antigens of all the groups studied to date[5,7,8]. How, or even whether, this T-cell response is involved in inducing an immune state is unknown. Finally, there are several unusual conditions of clinical hyper-responsiveness associated with filarial infections, such as tropical pulmonary eosinophilia (TPE)[9] and the Sowda form of onchocerciasis[10], that almost certainly result from immune responses to the parasite that are both quantitatively and qualitatively different from those seen in the other clinical conditions.

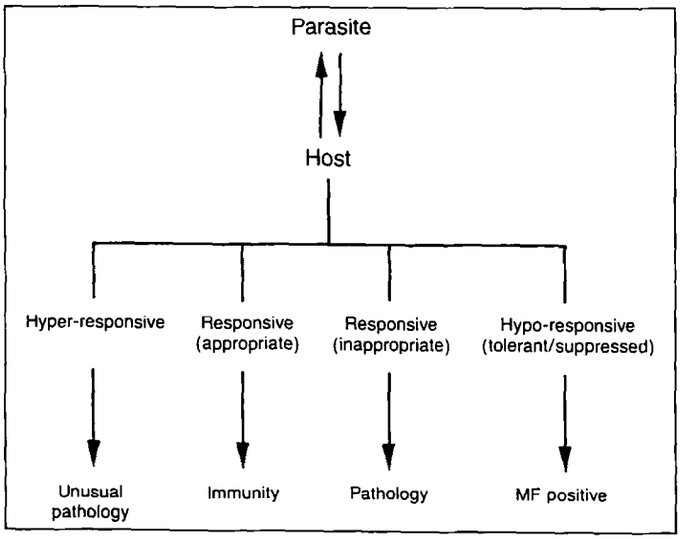

Fig. 1. *The relationship of the host's immune response and the consequences of infection.*

From the point of view of immunoregulation, there are two features of filarial infections that are particularly intriguing. First is the prominent immediate hypersensitivity response manifested by the marked elevation of blood IgE and eosinophil levels and second is the persistence of microfilariae in the blood or skin that is associated with minimal host immune responsiveness to these parasites. This review will examine the current understanding of the regulatory aspects of these responses.

Immediate hypersensitivity responses

IgE responses

Immediate hypersensitivity reactions are characterized by the presence of IgE antibody, eosinophils, mast cells

and basophils, each of which has been implicated in the resistance to infection and in the pathogenesis of the various clinical manifestations of infection[1]. High serum IgE levels are characteristic of filarial infections, although much of the IgE produced is not parasite-specific and is, therefore, considered to be polyclonal[11]. In fact, the proportion of the total IgE that is parasite specific differs in the various clinical states of lymphatic filariasis: the highest ratio of specific antibody is found in patients with TPE and the lowest in those with asymptomatic microfilaremia[11]. The induction of the IgE response in parasitic infections has clearly been shown to be T-cell dependent both in animal models[12] and in humans[13]. Extensive studies in the mouse system, both *in vitro* and in animal models of helminth infection, have shown that IL-4 is the cytokine primarily responsible for inducing IgE responses and that IFN-γ effectively blocks this IL-4 effect (see F. Finkelman *et al.*, this issue). The mechanism responsible for the induction of IgE by IL-4 appears to involve the regulation of isotype switching to IgE in uncommitted B cells[14].

Similar studies have also shown that IL-4 is essential for the generation of IgE in human filarial infections[15]. In these experiments, parasite antigens induce IgE production in cultures of peripheral blood mononuclear cells (PBMCs) from patients with filariasis; these responses are completely inhibited by the simultaneous addition of anti-IL-4 antibody[15]. Conversely, antibodies to IFN-γ augment the filarial antigen-stimulated IgE production *in vitro*; this result suggests that endogenously produced IFN-γ normally inhibits IgE production. More direct evidence for this hypothesis has come from experiments in which the addition of recombinant human IFN-γ *in vitro* has been found to inhibit both parasite antigen-induced and spontaneous IgE production in a dose-dependent fashion. Furthermore, the induction of IgE by filarial antigens is dependent on the concentration of antigen, with higher antigen concentrations actually suppressing IgE production and this suppression can be reversed by anti-IFN-γ antibody.

One interpretation of these data is that antigens in low concentrations specifically induce the production of more IL-4 than IFN-γ, whereas higher concentrations of the same antigens induce the production of relatively more IFN-γ, either by the same cells or by other populations of $CD4^+$ cells. Mechanistically, these observations suggest the presence of a subset of parasite-specific T cells that can induce IgE responses, which may be like T_H2 cells that produce IL-4 and IL-5 and which have a higher avidity for parasite antigens and are therefore preferentially activated by low antigen concentrations. At higher antigen concentrations additional $CD4^+$ cells, with lower avidity for parasite antigens that are predominantly IFN-γ producing will also be activated. It is well known that most $CD4^+$ cells produce IFN-γ (Fig. 2).

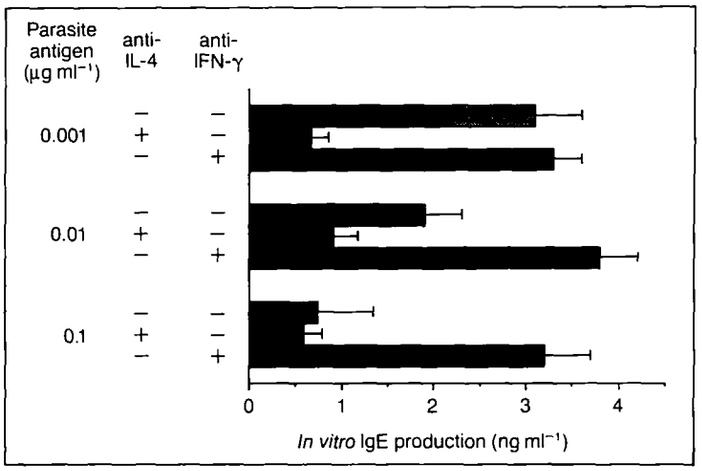

Fig. 2. *Effect of anti-IL-4 and anti-IFN-γ on the induction of IgE induced by different concentrations of parasite antigen. Each bar represents the mean ± SEM IgE production in cultures to which antigen alone was added (shaded bars), or antigen and anti-IL-4 (hatched bars), or anti-IFN-γ (dark bars). Data was obtained from Ref. 15.*

Eosinophil responses

The induction of eosinophilia, like IgE responses, has also been shown to be regulated by T cells[16] and, indeed, human IL-5 has been shown to be a potent and specific stimulus for eosinophil production *in vitro*[17]. The hypothesis that IL-5 is the most important of the eosinophilopoeitic cytokines is supported by the recent observation that mitogen stimulation of PBMCs from patients with filarial-associated eosinophilia induces large amounts of both IL-5 mRNA and protein IL-5 compared with mitogen-stimulated PBMCs from normal individuals[18]. In contrast, the production of IL-3 and granulocyte–macrophage colony-stimulating factor (GM-CSF), cytokines which are also associated with the induction of eosinophilia, was similar in the two study groups. Interestingly, some parasite antigen specificity was associated with the induction of the IL-5 response in that nonparasite antigens did not stimulate IL-5 in PBMCs from filaria-infected patients, but filarial antigens did (authors' unpublished data).

Allergic responses

In spite of the frequently elevated levels of IgE and eosinophils, individuals with filarial infections rarely have clinically apparent allergic reactions to these parasites, although their basophils and mast cells can be highly sensitized with specific anti-parasite IgE[19]. This effector arm of the immediate hypersensitivity response is thought to be modulated by the production of blocking antibodies that are abundant in the sera of filaria-infected patients. The antibodies, by definition, can inhibit IgE-mediated histamine release from basophils of filaria-infected patients[19]. They are predominantly of the IgG4 isotype[20] and, by immunoblotting, show a pattern of antigen recognition that parallels that of IgE. These findings suggest that blocking antibodies bind to parasite allergens in the fluid phase, preventing access of these allergens to the IgE-coated mast cells or basophils[21].

Although definitive proof is lacking, overall control of the blocking antibody response may occur at the T-cell level. For example, little is known about the cytokine regulation of IgG subclass expression in humans. However, the parallel elevations of IgG4 and IgE reported in filarial infections suggest similar regulatory mechanisms that perhaps involve IL-4 and IFN-γ. IL-4 has been shown to stimulate IgG4 *in vitro* in normal human PBMCs[22], whereas recombinant human IFN-γ can inhibit the spontaneous production of IgG1, IgG3 and

IgG4 but not of IgG2 (Ref. 23). The role of these cytokines in regulating parasite antigen-induced IgG subclass expression is unknown.

The IL-4- and IL-5-producing T-cell subset in humans

The increased production of both IL-4 and IL-5 by mitogen-stimulated PBMCs of helminth-infected individuals compared with uninfected individuals (D.O. Freedman, submitted) indicates a generalized increase in IL-4- and IL-5-producing lymphocytes among infected individuals. Although these observations suggest an expansion of filaria-specific T cells similar in character to mouse T_H2 cells, there are important differences. T-cell clones obtained from the PBMCs of individuals infected with filariasis and uninfected controls produce a range of different cytokines including IL-2, IFN-γ, IL-4 and IL-5 (Ref. 24); however, only a very small proportion of the clones exclusively produce IL-4 and IL-5. Recent studies *in vitro* have shown that repeated antigenic stimulation of human PBMCs can change T-cell phenotype and increase the production of IL-4 correspondingly, suggesting that the maturation from naive to memory T cells may influence the types of cytokine produced[25]. Hence, the chronicity of helminth infections may favor the expansion of T-cell subsets producing IL-4 and IL-5. This idea can be tested by recently developed assays that can directly measure the frequency of different cytokine-producing cells either by filter-spot ELISA or by *in situ* hybridization of samples from helminth-infected patients at different stages of their disease.

Immunological tolerance to parasite antigens

It is important to realize that by avoiding the host's immune responses, microfilaremia can persist and the chances of parasite transmission are maximized. Microfilaremic individuals have selectively impaired T- and B-cell responses to filarial parasite antigens, in contrast to the relatively strong cellular and humoral responses seen in individuals that are symptomatic and amicrofilaremic. This impairment is manifested by altered delayed-type hypersensitivity responses to intradermal application of filarial antigens[26], by a marked reduction in T-cell proliferation[2,4,5] as well as by reduced IL-2 and IFN-γ[27] production[6,27]. Microfilaremic patients also have altered B-cell responses, as indicated by both their relatively low serum levels of parasite-specific antibodies[3,12,28] and their impaired immunoglobulin production to parasite antigens *in vitro*[4]. The mechanisms underlying this immunological hyporeactivity remain poorly defined, although a number of possibilities have been proposed, including the development of immune tolerance[5], active suppression by monocytes[29], suppressor T cells[30] or soluble suppressive parasite products[29].

The immunomodulatory role of monocytes or suppressor T cells remains uncertain, especially as removal of monocytes or $CD8^+$ T cells fails to restore the poor lymphocyte proliferative responses generally seen in patients with *W. bancrofti* infections[5]. Furthermore, stimulation of PBMCs from microfilaremic patients with pokeweed mitogen (a T-cell-dependent B-cell mitogen) fails to induce parasite-specific immunoglobulin production, whereas a marked response to this mitogen can be elicited from PBMCs of amicrofilaremic patients[5].

Serum from microfilaremic individuals has been shown to suppress selectively the parasite antigen-induced lymphocyte proliferation from amicrofilaremic individuals[29], suggesting that active and specific immune suppression by filarial antigens may occur. In addition, subsequent characterization of filarial-derived proteins shows that high molecular mass extracts from microfilariae[31] and phosphocholine-containing antigens of adult *B. malayi*[32] can inhibit mitogen-induced lymphocyte proliferation. This suppression is a general phenomenon and is not specific for parasite-reactive lymphocytes.

In contrast to the concept of active suppression, it has been proposed that some individuals develop immune tolerance to filarial infections, either from prenatal or perinatal sensitization to parasite antigens[33]. This hypothesis is supported by the recent observation that microfilaremic individuals with *W. bancrofti* have markedly diminished precursor frequencies for parasite-responsive T and B cells compared with amicrofilaremic individuals[34].

Finally, there is probably a genetic regulation of the host response to parasite antigens[35,36] that may account for differential susceptibilities and clinical manifestations of the disease observed among individuals[37].

The use of defined antigens to study parasite-specific immune responses

One of the major obstacles to the study of parasite-specific immune responses has been the lack of well-defined parasite antigens. Indeed, most investigations of the immune responses in filarial infections have relied on crude soluble antigen preparations. To examine the relationship between the structure of the antigen and the immune response it engenders, recent studies have begun to characterize biochemically purified and recombinant antigens that can stimulate different arms of the immune system[38–40].

Preliminary studies of T-cell epitopes of recombinant *O. volvulus*[41] and *B. malayi* antigens[40] may help to define the nature of the antigenic signals necessary for the induction of the IgE and eosinophil responses. For example, there are several distinct B-cell epitopes on recombinant filarial paramyosin that have been shown to be a target in microfilarial clearance in mouse *B. malayi* infections[42]. These B-cell epitopes are found primarily at the amino-terminal end of the molecule. These epitopes are preferentially recognized by patients with *O. volvulus* infections[43] and immunoglobulin isotype analysis has revealed that they are recognized by IgE, IgG2 and IgG4 anti-paramyosin antibodies. These findings suggest that specific epitopes may be important in determining the nature of the subsequent immune response.

The effect of anti-parasite treatment on the immune response

Since practical, effective treatment of the adult parasites is currently unavailable for most filarial infections, treatment is primarily directed at the microfilariae but the response to treatment depends on the clinical manifestation of the filarial infection. Among patients with TPE, Sowda, filarial fevers, lymphangitis and clinical conditions without detectable microfilariae (Fig. 1), treatment results in marked improvement of their symptoms.

This improvement is likely to result from a reduction in parasite burden, and thus the sensitizing antigenic load.

In contrast, treatment of microfilaria-positive individuals with diethylcarbamazine (DEC) or ivermectin causes rapid death of the microfilariae and release of parasite antigens, and often results in acute inflammatory reactions that can be particularly severe. Microfilaremic individuals are often reluctant to seek therapy because of these reactions but treatment is essential to interrupt transmission of the parasite in the community. The immunopathology seen to develop within the first few weeks after treatment of microfilaremic patients includes increased levels of blood eosinophils[44,45], circulating eosinophil-derived proteins[46], phosphocholine-containing antigens in the serum[47] and circulating microfilarial excretory–secretory antigens[47]. Significant increases in serum anti-filarial IgG antibody levels also occur within two weeks of treatment[48], whereas filaria-specific IgE levels have been described as variable or unchanged. In some patients both IgG and IgE are produced in response to antigens previously unrecognized by the host[48,49] and such antibodies are more likely to occur in patients with greater microfilarial densities[48]. It is possible that these antigens have remained hidden from the host immune response, thereby contributing to the persistence of infection and once therapy is given, these antigens become exposed and evoke a measurable immune response.

Longitudinal studies before and after anti-microfilarial chemotherapy have also been used to study the effect of microfilariae and their products on T-cell reactivity. Specific increases in T-cell responses to filarial antigen have been observed 3–6 months after treatment in microfilaria-positive patients with *B. malayi*[50], *W. bancrofti*[49] and in *O. volvulus* infections (C. Steel and T.B. Nutman, unpublished). When lymphocyte proliferation was examined earlier (two weeks after treatment), a generalized immunosuppression was observed[4], resulting perhaps from the release of phosphocholine-containing or other suppressive microfilarial antigens[47]. These and similar studies have been interpreted as supporting the hypothesis that microfilaria-derived products are important in suppressing lymphocyte reactivity[38], although additional verification of this conclusion is clearly needed.

Conclusions

In this review, the potential mechanisms responsible for immediate hypersensitivity responses in filarial infections have been examined by emphasizing the interactions between the different subsets of parasite-specific T cells and the roles played by the cytokines they produce. A clear understanding of these interactions is critical for defining the regulatory controls of the IgE and eosinophilic responses in parasite-infected individuals. It seems possible that immediate hypersensitivity responses are important effector mechanisms for eliminating the parasite.

Individuals with persistent microfilariae may fail to mount adequate immediate hypersensitivity responses as a result of either (1) active suppression of the lymphocytes that generate the signals (for example IL-4 and IL-5) that are necessary to produce such responses, or (2) the absence or failure of lymphocytes that respond to parasite antigens. This view is supported by the observation that individuals with lymphatic filariasis have persistent microfilaremia, lower eosinophilia and parasite-specific IgE and IgG levels, compared with most amicrofilaremic patients with chronic lymphatic obstruction or TPE.

The role of immediate hypersensitivity responses in immunity is less clear and will require a more detailed understanding of the control of antigen-specific lymphocyte and immunoglobulin responses among individuals with different clinical manifestations (see Fig. 1) and of infected individuals before, during and after treatment. An understanding of these mechanisms will have important implications for immunological intervention to control the serious human morbidity that these widespread helminth infections cause. Such understanding will also provide insight into basic immunological issues such as tolerance, T- and B-cell differentiation and networks of T-cell interactions that have significant implications for other diseases.

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Helper T-cell subsets in mouse leishmaniasis: induction, expansion and effector function

Richard M. Locksley and Phillip Scott

It has been a surprise to find that the two distinct subsets of mouse CD4+ T cells identified from clones cultured in vitro *also occur during* Leishmania *infection. The spectrum of disease encountered during these infections ranges from successful resolution to fatal dissemination and in mice these outcomes are accompanied by expansion of T_H1 or T_H2 CD4+ cells, respectively. This review focuses on the mechanisms that cause such disparate responses to the parasite.*

Leishmania are dimorphic protozoa that cause disease in humans and other animals on all continents except Australia and Antarctica. Inoculation of inbred strains of mice with *Leishmania major* results in a spectrum of responses, from control and resolution of infection to progressive and fatal disease, that mimic the responses in human cases. In resistant strains of mice, such as C57BL/6 or C3H/HeN, control of infection is associated with expansion of gamma-interferon (IFN-γ)-producing T cells in the draining lymph nodes and the appearance of delayed-type hypersensitivity to parasite antigens[1]. Conversely, progressive infection in susceptible strains, such as the BALB/c strain, is associated with the appearance of IL-4-producing T cells and hyperglobulinemia, including elevated levels of IgE[2]. Evidence to date suggests that these different lymphokine profiles are the result of the activation of either T_H1 cells that mediate healing, or T_H2 cells that mediate progressive infection[3,4]. Interestingly, the response of BALB/c mice is not due to deletions in the T-cell repertoire, since a variety of immunomodulatory interventions, including prior sublethal irradiation[5], prior CD4+ cell depletion[6] and therapy with anti-IL-4 (Ref. 7), enable these mice to heal subsequent *Leishmania* infection; each of these procedures is associated with ablation of the predominant T_H2-cell response that occurs in untreated mice. The ability to modulate such strikingly biased subset responses has created intense interest in this model of spectral infectious diseases.

Induction of T_H subsets

The initial events that induce the maturation of distinct T_H-cell populations during *Leishmania* infection remain unknown. Within 72 h of infection, the draining lymph node cells of BALB/c mice produce a biased T_H2 response, characterized by the generation of IL-4 and IL-5 after antigen stimulation *in vitro* (P. Scott, unpublished). In contrast, cells from previously immunized BALB/c mice or resistant C3H mice generate IFN-γ. Although the contributions made by CD4− cells have not been extensively evaluated, these experiments suggest that the induction of T_H subsets may occur very rapidly, a concept