

Lymphatic filariasis: parallels between the immunology of infection in humans and mice

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SUMMARY

Mouse models of Brugia infection have provided much useful quantitative and qualitative information on the immune response elicited by different life cycle stages of filarial worms. Many parallels exist between the immune response in the mouse and the infected human and in this review we highlight areas of topical interest, including the induction of specific cytokine responses and their role in immunomodulation and protective immunity. These studies have reinforced the concept that different life cycle stages of filarial parasites each have their own mechanism of modulating responses so that potentially inflammatory IFN- γ responses are downregulated. While the precise mechanisms of protective immunity remain to be defined, studies in the mouse have suggested novel pathways, including a possible role for granulocytes.

Keywords antibodies, *Brugia*, filariasis, granulocytes, IFN- γ , IL-4, IL-5, IL-10, protective immunity, T regulatory cells, neutrophils

INTRODUCTION

Lymphatic filarial worms are complex multicellular organisms which have evolved numerous mechanisms of evading the host immune response and promoting their own survival. These parasites are long-lived in their human hosts with an average reproductive life span in the order of 6 years. In infected humans, the immune response is dominated by the downregulation of antigen-specific proliferative responses and interferon (IFN)- γ -producing cells and the upregulation of interleukin (IL)-4-driven immunoglobulin (Ig)G4 antibody responses (1–4). The mechanisms which give rise to these phenomena, their relationship to each other and their impact on both host and parasite remain the subject of much discussion. It is tempting to describe the immune response in lymphatic filariasis in terms of the Th1/Th2 paradigm, but recent studies in infected humans demonstrate a greater level of complexity (5,6) and suggest that applying a Th1/Th2 paradigm to human infection may be inappropriate.

During the course of natural infection, humans are simultaneously exposed to multiple life cycle stages [microfilaria (mf), adults, L3 and developing larvae] making it difficult to distinguish the relative roles of different life cycle stages in inducing or modulating particular immune responses. Moreover, in the human host, individual life cycle stages inhabit different anatomical compartments and are therefore likely to encounter distinct subsets of antigen presenting cells (APC) which may also modulate responses in a stage-specific manner. Mouse models of infection provide the opportunity to study the role of individual life cycle stages in eliciting defined responses and offer the possibility of dissecting the mechanisms by which these responses arise. While studies in mice are beginning to shed light on the mechanisms by which filarial parasites drive IL-4 production, much debate still focuses on the relevance of IL-4 for parasite and host (7,8). The recent description of T regulatory cells (Tr1/Th3) which produce IL-10 and transforming growth

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factor (TGF)- β in a mouse model of autoimmune colitis (9) necessitates a re-examination of the mechanisms by which antigen-specific responses may be downregulated in filariasis and the role of these cytokines in the mouse model is starting to be elucidated. Finally, the recent observation that IL-4 and IL-5 are independently regulated in human filariasis (6), in combination with studies in mice which indicate that IL-5 can act alone in host protection (10–12), has important consequences for protective immunity to filariasis. In this review, we discuss recent developments in human filariasis and relate these observations to studies in mice. We show that perhaps surprising parallels exist between the two host-parasite systems and that focusing attention on conundrums of the mouse model can enlighten some of the more complex aspects of human filarial biology.

THE ROLE OF ELEVATED IL-4 LEVELS IN LYMPHATIC FILARIAL INFECTION

Most studies in filarial-infected humans have shown that stimulation of peripheral blood mononuclear cells (PBMC) with filarial antigen or with polyclonal stimulants results in high levels of IL-4 production (4,13). Somewhat surprisingly, however, closer examination of the published data on *Brugia malayi* infection suggests that there is little difference in IL-4 levels between the different clinical groups found in an endemic area. PBMC from endemic normals (EN), who are presumably uninfected and elephantiasis patients (CP), who are likely to have cleared their original infection, secrete levels of IL-4 equivalent to actively infected microfilaraemics (mf⁺) (6,13). Similarly, in *Wuchereria bancrofti* infection, no differences are observed in the frequency of IL-4 producing cells as assessed by ELISPOT (14), or in the IL-4 mRNA levels between mf⁺ and CP patients (15,16). However, a significant difference is observed if the ratio of IL-4 to IFN- γ -producing lymphocytes in mf⁺ is compared with that of CP patients, suggesting that the relative levels of cytokine production (IL-4 : IFN- γ) may be a factor in determining the outcome of the immune response (14). Several frequently posed questions take on a new bearing in the light of these observations: Which developmental stages of the parasite elicit IL-4 production if high levels are found in all clinical groups? Are depression of proliferative and IFN- γ responses in mf⁺ individuals linked to or independent of IL-4 production? Does IL-4 play any role in protective immunity?

Different life cycle stages elicit IL-4 production

Studies in different inbred strains of mice have shown that the L3 elicits a significant IL-4 response within 12–14 days of infection, irrespective of the route of infection (i.p., s.c.

or i.v.) (8,17). This response is apparent even in those mice not classically considered to be strong Th2 responders (e.g. C57Bl/6), suggesting that the L3 is a very potent stimulator of IL-4. Until recently, it was unclear whether L3 elicited an IL-4 response from the outset of infection or whether Th1 cells were activated and subsequently downregulated. Examination of the responses immediately following infection of BALB/c mice with L3 of *B. pahangi* revealed that a burst of IL-4 transcription occurs in the draining lymph node within 24 h of infection. This early IL-4 is derived from an unusual population of double-negative (DN) CD4⁺ CD8⁻ $\alpha\beta$ T cells and is specific to infection with the L3, as infection with mf has no such effect (8). Extrapolation of these results from the mouse to human suggests that continual exposure to L3 in endemic areas will prime the immune system for IL-4 production. Indeed, as all people in an endemic area are exposed to L3, this may provide an explanation for the high levels of IL-4 found in all clinical groups. IL-4 production is a hallmark of many different helminth infections and it is interesting to speculate whether these parasites employ a common mode of activation of IL-4-producing cells upon primary infection, in a similar way to the pattern recognition motifs of many prokaryotic organisms which result in induction of Th1 cells (18). While the range of different cell types that have been implicated as producers of early IL-4 following exposure to helminth antigen (e.g. eosinophils, $\gamma\delta$ T cells and conventional CD4 T cells, in addition to DN T cells) possibly argues against recognition of a common motif, further studies are required to clarify the mechanism of IL-4 induction (19–21). For example, it would be interesting to determine whether helminth parasites that enter the body via a particular route (the skin, the gastrointestinal tract, etc.) elicit IL-4 from a common source.

If L3 prime the immune response for IL-4 production, what then is the role of the adult parasite in driving IL-4? Implantation of BALB/c mice with adult worms clearly elicits optimal levels of IL-4 (7) and recent studies have demonstrated that the APC have a critical role in driving IL-4 production from T cells. For example, Loke *et al.* (22) demonstrated that naive T cells differentiated into IL-4 producing cells when exposed to peritoneal macrophages derived from mice implanted with adult *B. malayi*. Interestingly, a specific secreted product of adult filarial nematodes (the phosphorylcholine containing glycoprotein, ES-62) has been shown to induce murine dendritic cells to elicit the differentiation of naive T cells to type 2 responses (23).

In humans, it is not possible directly to assess the role of the adult worm in eliciting IL-4; by the time adult worms develop, infected humans have already been exposed to L3 and developing L4 and the immune response is presumably already skewed. As referred to above, in most studies where

attempts have been made to correlate IL-4 levels with clinical status, no differences have been reported between groups so that patients who are probably negative for adult worms (EN or CP) have equivalent levels of IL-4 to those with adult worms (mf⁺) (13). These results hold true even for studies in which the presence of adult worms has been confirmed by circulating antigen tests (15). However, additional studies are required in well-characterized human populations properly to dissect the role of adult worms in IL-4 production. From studies in the mouse, it appears that the L3 may be the primary stimulus for IL-4 production and that continued exposure to L3 maintains elevated levels of this cytokine (8,17,24), while the adult worms have evolved separate/distinct mechanisms for skewing the immune response.

The role of mf in eliciting IL-4 responses is equally complex to dissect. Several studies have demonstrated that, in contrast to L3, infection of a variety of mouse strains with mf, whether by the s.c., i.p. or i.v. route, induces an IFN- γ response at early time points postinfection (7,25–27). However, the early IFN- γ production is followed by an increase in levels of IL-5 and a subsequent decline in IFN- γ (25). A similar switch in cytokine production was observed when mice were given multiple immunizations with mf extract (28). More recent studies, in which mice were subjected to high levels of live mf or to multiple live mf infections by the intravenous route, have demonstrated that chronic exposure elicits high levels of IgE and a switch in cytokine profiles from IFN- γ to IL-4 (27). Thus, mf also appear to have the capacity to induce Th2 responses, although the mechanisms by which the Th1–Th2 switch occurs have not been studied. One intriguing possibility to explain these results is that mf may elicit apoptosis of the IFN- γ producing population, thus allowing the outgrowth of Th2 cells. We have recently observed that infection of BALB/c mice with *B. pahangi* mf results in significant levels of apoptosis both *in vivo* and upon restimulation with parasite antigen *in vitro* (29). Because several studies have documented that Th1 cells are more susceptible to apoptosis than Th2 cells (30), the switch in cytokine profiles in chronically infected mice may relate to increased levels of apoptosis in the Th1 cell population. In conclusion, studies in the mouse show that all life cycle stages to which the human host is exposed have the potential to drive IL-4 production; however, each stage may utilize a separate mechanism to do so.

Does IL-4 have a role in the downregulation of proliferative and/or IFN- γ responses?

Although the immune response in infected/exposed individuals is dominated by production of IL-4, the exact role of

this cytokine in lymphatic filariasis remains controversial. Examination of proliferative and cytokine responses pre- and postchemotherapy of *B. malayi* patients with diethylcarbamazine (DEC) has demonstrated that IL-4 levels remain unchanged following clearance of mf (and adults?) while both the proliferative response and IFN- γ production were restored post-treatment (31). The interpretation of these results is somewhat complicated by the uncertainty of whether adult parasites, in addition to mf, were removed by DEC. However, in a study from Haiti with *W. bancrofti*-infected individuals, treatment with ivermectin, which is not macrofilaricidal, restored proliferative responses to filarial antigen (32). These results support a direct association between the presence of circulating mf and the suppression of proliferative responses. Furthermore, the Sartono study suggests that both the downregulation of proliferative and IFN- γ responses are regulated independently of IL-4 (31). In addition, the consistently lower levels of IFN- γ found in mf⁺ patients have not been directly linked to higher IL-4 levels in these patients (6,13). Indeed, some studies which have sought a direct role for IL-4 in proliferative suppression have proved negative. Using PBMC from *B. malayi*-infected humans, attempts were made to reverse the proliferative defect with a range of immunomodulators and neutralizing Ab (33). Neutralization of IL-4 (or IL-10) had no effect on the antigen-specific proliferative defect (33).

A variety of experiments in the mouse model of *Brugia* support a role for IL-4 in modulating proliferative and IFN- γ responses and are beginning to shed some light upon the mechanisms involved. In mice implanted i.p. with adult *B. malayi*, peritoneal exudate cells (PEC) are unable to support the proliferation of a conalbumin-specific T cell clone, although cytokine secretion is not impaired (34). The generation of these defective APC is dependent on IL-4, but not IL-10 (35); however, the ability of these APC to block proliferation is IL-4-independent (36). In other experiments in which BALB/c mice were infected with L3 by the s.c. route, polyclonal (but not antigen-specific) proliferative and IFN- γ responses were suppressed (26). Neutralization of IL-4 *in vitro* resulted in a partial restoration of the polyclonal proliferative response, and increased the IL-2 and IFN- γ levels to mitogen but had no effect on antigen-specific cytokine production (26). However, priming of antigen-specific T cells in IL-4 knockout mice does result in the production of filarial antigen-specific IFN- γ suggesting that IL-4 is particularly important in initiating differential T cell development (17,37). Once established, IL-4 responses have been shown to be difficult to reverse both in filarial models and in other mouse model systems, while IFN- γ responses are more easily overridden (27,38). In an interesting parallel to these findings, in areas of high transmission, the

proliferative defect in mf^+ patients is not only antigen-specific, but also extends to mitogens and unrelated antigens and is associated with high serum IL-4 levels (39). Thus, under certain conditions (L3-infected mice and hyperinfected humans) a profound suppression of polyclonal responses can result from filarial infection. In the mouse, IL-4 plays a role in downregulating both polyclonal proliferative and IFN- γ responses. It remains to be seen whether IL-4 also plays an equivalent role in the suppression of polyclonal responses in hyperinfected humans.

IL-10 AND TGF- β ARE IMPLICATED IN PROLIFERATIVE SUPPRESSION

The partial role of IL-4 in proliferative suppression during filarial infection suggests that other downregulatory pathways may be equally, if not more, important in controlling cell division. Interestingly, studies using PBMC from *W. bancrofti*-infected individuals have implicated both IL-10 and TGF- β in downregulating proliferative responses (14). In a small group of mf^+ patients, both IL-10 and TGF- β were upregulated compared to CP patients and neutralization of either cytokine resulted in increased levels of proliferation in PBMC of the mf^+ but not the CP patients (14). Re-examination of these data in the light of recent studies on T regulatory cells (Tr1/Th3) (40) suggests that this population of cells may be expanded in mf^+ individuals and may contribute to the downregulation of proliferative and pro-inflammatory responses. Indeed a recent study in *Onchocerca volvulus* infection showed that simultaneous neutralization of IL-10 and TGF- β augmented antigen-specific proliferation in mf^+ individuals, and in addition, four Tr1 T cell clones were obtained from a mf^+ patient (41).

In other studies, neutralization of IL-10 alone, in cultures of PBMC from *W. bancrofti* mf^+ individuals, has been shown to restore the proliferative response and augment IFN- γ responses to filarial antigen (42). In one study, a significant correlation between the level of expression of IL-10 mRNA and the reduction in parasite-specific proliferative capacity was observed (16). Induction of IL-10 by filarial nematodes may therefore be a critical factor in the enhancement of mf survival. In support of this hypothesis, recent experiments in the *B. malayi*-mouse model showed that both CBA/Ca and C57Bl/6 mice treated with anti-IL-10 clear intravenous mf infections more rapidly than control mice. In addition, IL-10-deficient mice (C57Bl/6) implanted i.p. with adult worms clear mf , but not adult nematodes, more rapidly than wild-type mice (Gray *et al.*, unpublished data).

Although, the induction of Tr1 cells in lymphatic filariasis is an appealing hypothesis to explain the phenomena of cellular hyporesponsiveness, several lines

of evidence suggest that the 'suppressive' cell population may not, in fact, be a T cell. High IL-10 production by PBMC from filarial patients is frequently spontaneous and occurs in the absence of antigenic stimulation: such production can be as high as 570 pg/ml in mf^+ patients (43). This spontaneous production suggests that the primary source of IL-10 may be an APC and not a T cell. In agreement with this, studies in a mouse model of proliferative suppression have shown that irradiation of the splenic APC population or replacement of the APC with those from naive mice can restore both the *in vitro* polyclonal proliferative and type 1 cytokine responses of spleen cells taken from L3-infected mice. Furthermore, antibody neutralization of IL-10 is also effective in restoring antigen-specific type 1 cytokine responses (44), although, as yet, there is no evidence of a direct association between APC and IL-10 in this model. Thus although Tr1 cells may play a role in filarial proliferative suppression it is likely that downregulatory cytokines produced by APC will prove at least as important in the initiation and maintenance of the tolerant state. Indeed, in several model systems, it is now well-documented that APC producing IL-10 can downregulate type 1 responses while inducing type 2 responses (45,46). The role of TGF- β in experimental models has been hampered by a paucity of reagents. However, a recent report showed that TGF- β , but not IL-10, derived from peritoneal-exudate macrophages from filarial-implanted mice is involved in the differentiation of naive T cells to type 2 cells (22). In addition, IFN- γ production is restored in the presence of anti-TGF- β (22). It is likely that different cell types can downregulate proliferative responses and/or IFN- γ responses by different mechanisms and that the interplay of these cell types and the relative importance of IL-10 or TGF- β production will vary in a site-specific manner.

ANTIGEN-STIMULATION OF IL-10

Several observations point to the fact that particular antigens may stimulate IL-10 production and the consequent suppression of proliferative activity. For example, the source of antigen used for *in vitro* restimulation of PBMC from filarial patients is an important determinant of cellular reactivity (47). PBMC from mf^+ patients proliferated in response to adult male antigen but not to antigen derived from mixed sex adults or mf , implying that the proliferative defect is specific to mf -derived antigen. In addition, as mentioned earlier, PBMC from these mf^+ patients secrete significantly higher levels of IL-10 compared to cells from CP patients when stimulated with mixed sex adult antigen or mf antigen. These differences in IL-10 production were much less pronounced when PBMC were restimulated with adult male antigen (47).

The nature of the filarial antigens responsible for eliciting IL-10 has not yet been fully determined.

Early studies, in which filarial extracts were fractionated according to size, demonstrated that IL-10 is induced by some antigens but not others (48). More recently, the increasing use of recombinant antigens from filarial nematodes has shown that certain types of antigen specifically elicit IL-10. For example, a cysteine protease inhibitor (Av17) secreted by adult females, but not males or mf, of the filarial nematode *Acanthocheilonema viteae*, inhibits polyclonal and antigen-specific proliferation of murine T cells in a partially IL-10-dependent manner (49). Interestingly, Av17 also increases the spontaneous and antigen-driven production of IL-10 by splenocytes. It remains to be seen whether the recently identified serine protease inhibitor of *B. malayi* mf can modulate cytokine responses in a similar fashion (50). Another *A. viteae* secreted product, ES-62, which has a homologue in *B. malayi*, is a phosphorylcholine-containing glycoprotein. Apart from potentially interfering with the cell-signalling machinery required for cellular proliferation, ES-62 is also capable of inducing IL-10 production from naïve spleen cells (51). Also intriguing in this battery of filarial molecules with potential immunomodulatory properties is the recent identification, in both *B. malayi* adult nematodes and mf, of a filarial homologue of mammalian TGF- β (52).

In another parasitic helminth, *Schistosoma mansoni*, oligosaccharides present on eggs and schistosomulae can directly stimulate mouse peritoneal B1 cell outgrowth and IL-10 production (53,54). Although oligosaccharide stimulation of IL-10 by filarial nematodes has not yet been addressed, the role of B1 cells in filariasis has been studied indirectly in a variety of filarial models using mice with mutations in the *btk* gene (*xid* defect) (55–57). These mice, amongst other defects, are deficient in the B1 cell lineage. The survival of several filarial nematodes, including *B. malayi* and *B. pahangi* L3 and mf, *L. sigmodontis* L3 and *Setaria digitata* mf, is greatly enhanced in these mice. As yet, the exact mechanism for this increased susceptibility to filarial infection is unclear, in part due to the significant lesion created by mutation of *btk* in signal transduction. Thus, while *btk*-deficient mice lack B1 cells, *btk* is also expressed in both B cell and myeloid lineages and is known to be involved in B cell stimulation via CD40, CD38, IL-5 and IL-10. Significantly, *xid* mice have reduced IL-10 production and raised type 1 responses in addition to the more well-documented defects in antibody production. Mukhopadhyay *et al.* (56) showed that transfer of wild type macrophages, but not B cells, could reverse the susceptible phenotype of *xid* mice to *S. digitata* mf with a parallel reduction in type 1 responses. However, more precise experiments are required to determine whether macrophage-produced IL-10 downregulates type 1

responses and skews the response toward type 2 which is then capable of killing parasites. An alternative explanation may be that lower IL-5 responses in *xid* mice allow increased parasite survival (see forthcoming section).

ARE TYPE 2 CYTOKINES IMPLICATED IN PROTECTIVE IMMUNITY?

While mouse models are extremely useful for ascertaining the different mechanisms by which immune responses are induced and modulated, they are central for the determination of potentially protective immune responses. As alluded to earlier, most human investigations have demonstrated little difference in IL-4 levels between different clinical groups, thus, the role of IL-4 in protective immunity in human filarial infections is in doubt. In addition, few studies have yet examined the presence of IL-13, a cytokine which has overlapping functions with IL-4, in different clinical groups.

In mouse models, two analyses of primary L3 infections in IL-4 knockout mice [(129 \times C57Bl6) or BALB/c] failed to confirm a role for IL-4 in parasite clearance (17) (Devaney *et al.*, unpublished data). In contrast, a recent study reported that the survival of *B. malayi* L3 is enhanced in IL-4 knockout mice (BALB/c) (58). These studies in IL-4 knockout mice are complicated by the presence of IL-13 which could compensate for the lack of IL-4. We have recently found that mf, L3, adult males and adult females of *B. malayi* all stimulate high levels of IL-13 *in vivo* (Lawrence, unpublished data; Devaney *et al.*, unpublished data). Moreover, *in vitro* stimulation of splenocytes from IL-4 knockout mice (129 \times C57Bl6) infected with each of these single developmental stages produced high levels of IL-13, albeit at lower levels than those found in wild-type mice (Lawrence, unpublished data). Thus, IL-13 could indeed partially compensate for the lack of IL-4 in these mice. Our preliminary experiments in IL-13 knockout and (IL-4 + IL-13) double knockout mice (129 \times C57Bl6), show that the survival of *B. malayi* L3 is enhanced only in the absence of both cytokines. In addition, mf recovery from (IL-4 + IL-13) double knockout mice implanted with adult nematodes is significantly greater than in wild-type mice. From this work, it is tempting to speculate that the presence of IL-4 and IL-13 is needed for clearance of a primary infection with the L3 or mf of *Brugia* sp. and that the relative importance of these two cytokines may vary between mouse strains.

DISSOCIATION OF IL-5 PROTECTIVE RESPONSES FROM IL-4 (AND IL-13?) RESPONSES

A recent study which investigated cytokine production as a function of clinical status and age demonstrated a complex

relationship between IL-4, IL-5 and IFN- γ levels and indicated that the classification of responses into type 1 or type 2 is not appropriate in this chronic disease (6). In this study, no differences were observed in IL-4 levels between mf⁺ and mf⁻ individuals; however, both IL-5 and IFN- γ levels were downregulated in mf⁺ patients (6). Similarities exist between this observation and studies of onchocerciasis patients in which downregulation of IL-5 and IFN- γ /IL-2 was observed in mf⁺ patients in comparison to mf⁻ patients, while IL-4 was not detected in either group (59,60). Recent work in two different mouse models exemplifies the fact that a dichotomy between IL-4 and IL-5 responses exists (10,61) and, in filariasis, this could have important consequences for both the host and the parasite.

IL-5 has been shown to be important in immunity in a number of filarial nematode model systems. Indeed, it is emerging from work in *Onchocerca* sp. and *L. sigmodontis* that IL-5 is an essential component of protective immunity in mice (10–12,62). Injection of *Onchocerca* mf into IL-4 deficient mice (C57BL/6) treated with anti-IL-5 showed that protection against primary and challenge infections is dependent on IL-5, but not IL-4. In addition, mf recoveries from μ MT (C57BL/6) mice were unaffected, suggesting that IL-5 mediates its effect via eosinophils independently of antibody (10). Protection against challenge infection of *L. sigmodontis* L3 following vaccination with irradiated larvae is also greatly impaired in IL-5 deficient mice while primary L3 infections are unaffected (12). Since few differences were observed in the level of parasite-specific antibody isotypes between wild type and IL-5 knockout mice, it is again likely that the absence of eosinophils, and not defects in B cell development, are responsible for these differences in protective immunity. Interestingly, a recent study has also shown that anti-IL-5 treatment can increase adult *L. sigmodontis* survival, although not early adult development, in primary infections and that this effect is negatively correlated with the eosinophil-dependent recruitment of neutrophils (11).

In contrast to the above results, other studies have implicated both IL-4 and IL-5 in protective immunity to *O. volvulus* L3 in BALB/c mice (62). In that study, increased levels of IL-5 and eosinophils were seen in both IL-4 deficient mice and in mice treated with anti-IL-4, suggesting that stimulation of eotaxin and increase of eosinophil responsiveness to β -chemokines by IL-13 alone is sufficient for eosinophil recruitment but perhaps not activation (62,63). Intriguingly a recent study in (IL-4 + IL-13) deficient mice has also shown that IL-5 production is induced, even in the absence of both these key type 2 cytokines, against the gut-dwelling nematode, *Nippostrongylus brasiliensis* but not against the tissue-dwelling

parasite, *S. mansoni* (61). Our preliminary experiments in which a heavy eosinophilic infiltration enters the peritoneal cavity of IL-13 knockout and (IL-4 + IL-13) knockout mice infected with adult *B. malayi* compared to wild-type mice (129 \times C57BL/6) further suggest that eosinophil recruitment is not dependent on these type 2 cytokines (Lawrence *et al.*, unpublished data). Thus, depending on the antigenic stimulus, IL-5 may be less linked to the development of Th2 cells than has previously been thought. In mouse models of lymphatic filariasis, the role of IL-5 has not been extensively examined, although a preliminary study demonstrated a significant decrease in protection following vaccination with irradiated larvae of *B. pahangi* in anti-IL-5 treated mice (Bancroft and Devaney, unpublished). Taken together, however, the above data show that IL-5 responses are frequently segregated from those of IL-4 and also IL-13, and thus the importance of the observed specific downregulation of IL-5 in lymphatic filarial patients should not be underestimated because it may indeed be a highly evolved mechanism of immune evasion by the parasite.

INFLAMMATORY MEDIATORS IN PROTECTIVE IMMUNITY

In studies where stringent criteria have been applied to the definition of endemic normals (for example detection of circulating antigen or parasite DNA), human immunity is reported to be correlated with higher levels of IFN- γ and IL-2 (15,64). The observed elevation of IFN- γ responses in EN and patients with chronic pathology (13) compared to patients with active mf⁺ infection has led to speculation that type 1 inflammatory responses may be involved in parasite killing. Activation of macrophages by IFN- γ could lead to parasite killing either by antibody-dependent cytotoxicity, resulting in the release of reactive oxygen intermediates or directly by the generation of reactive nitrogen intermediates.

In the mouse model, this has been investigated in a number of laboratories both *in vivo* and *in vitro*, however, the results have proved contradictory. *In vitro* experiments have generally shown that *Brugia* mf and adults are relatively resistant to killing by reactive oxygen intermediates such as hydrogen peroxide (65,66). Reactive nitrogen intermediates released by activated macrophages are however, effective in killing both mf and adult parasites (66,67) and peroxy-nitrite rather than nitric oxide appears to be more potent in this regard. A recent study in IFN- γ knockout mice suggests that the absence of IFN- γ can increase the recovery of infective larvae in mice (58). In contrast, we did not find a difference in the survival of a primary L3 infection in IFN- γ RKO (129) mice (68). Our

studies of microfilaraemia in CBA/Ca mice which rapidly clear mf infection, within 10 days, similarly show that administration of anti-IFN- γ is ineffective in blocking parasite killing. In mouse strains that support microfilaraemia for longer time-periods (69), the absence of either IFN- γ [IFN- γ KO (C57BL/6)] or the IFN γ R [IFN- γ RKO (129)] were ineffective in increasing mf survival (68).

Intriguingly, as mentioned earlier, we have recently discovered that IL-10 is also intimately involved in the regulation of a microfilarial killing mechanism. The mechanism for increased mf killing in IL-10-deficient mice is not yet established. However, in the absence of IL-10, IFN- γ mediated responses are highly upregulated, suggesting that an IFN- γ mediated responses such as nitric oxide may be involved. Nitric oxide mediated mechanisms have been shown to be capable of killing mf *in vitro* and L3 *in vivo* (although not at physiological doses) (66,70). An alternative and more intriguing mechanism, however, is linked to the role of IL-10 in the recruitment of granulocytes. Several reports have shown that, depending on the antigen used, the absence of IL-10 can profoundly effect recruitment of either eosinophils or neutrophils to the site of inflammation (71). Indeed, in our studies, absence of IL-10 and increased mf death is linked to highly upregulated neutrophilia which is also in accord with a previously mentioned study in which increased adult *L. sigmodontis* death correlated with IL-5 production and neutrophil presence (11).

Elucidation of the role of granulocytes as both effector and immunomodulatory cells is currently undergoing a renaissance in a number of model systems of infectious disease (72–74). Increasingly, the importance of these cells as first recruits to the site of infection and inflammation is becoming apparent and their ability to produce a variety of cytokines and chemokines has been shown to determine susceptibility or resistance in several models (72,73). In fact neutrophils, which are classically associated with inflammatory responses, have been shown to be a necessary factor in the development of type 2 responses against *L. major* infection (75). Clarification of the role of granulocytes and nonlymphoid cells in mouse models of filarial infection will further increase our understanding of the regulation and recruitment of protective effector cells and may illuminate the mechanisms involved in the differentiation of immune responses in filarial infection.

CONCLUSIONS

The mouse model of *Brugia* infection has been extremely useful for a range of studies on the immunomodulatory capacity of filarial nematodes and for studies aimed at defining the mechanisms by which filarial nematodes elicit

protective immune responses. Despite the fact that mice are not fully susceptible to infection with *Brugia* sp., the survival (and development) of specific life cycle stages for considerable periods of time provides an amenable experimental system. As we have attempted to highlight in this review, there are many parallels between the results obtained in the mouse model and those in infected humans. The ability to expose mice to single life cycle stages helps to define the role of specific stages in the human infection: for example, the role of the L3 in eliciting IL-4 in the mouse has been borne out by recent studies in the human population showing the relationship between IL-4 and transmission intensity. Moreover, recent studies in the mouse have suggested possible mechanisms of suppression of proliferation that could be investigated in the infected human. The role of cells other than T lymphocytes in steering the immune response in filariasis is now being appreciated and it is likely that mouse models will play an important role in elucidating the complex interactions of various cell types. In addition, the ever-increasing availability of gene knockout mice should allow more precise definition of the molecules and signalling pathways which filarial nematodes have subverted to enhance their survival and transmission.

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