

# Regulation of the immune response in lymphatic filariasis: perspectives on acute and chronic infection with *Wuchereria bancrofti* in South India

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## SUMMARY

*Delineating the immune responses in lymphatic filariasis has been complicated not only by the rapidly expanding knowledge of new immunological mediators and effectors, but also by new methodologies (in particular, circulating filarial antigen detection) for defining and categorizing filarial-infected individuals. By using assays for circulating antigen in the sera collected as part of the many immunological studies performed on individuals in a Wuchereria bancrofti-endemic region of South India, we have attempted to explore the influence of patency on the antigen-driven proliferative and cytokine responses seen in peripheral blood mononuclear cells of individuals with varying clinical manifestations of lymphatic filarial infection. Moreover, we have provided perspectives on the differences between acute and chronic infection with W. bancrofti and suggested mechanisms that may underly the modulation of the immune response as patency occurs.*

**Keywords** lymphatic filariasis, *Wuchereria bancrofti*, tolerance, circulating filarial antigen, cytokine, tropical pulmonary eosinophilia

## INTRODUCTION

Of the 129 million people currently thought to be infected with the three lymph-dwelling filariae of humans, *Wuchereria bancrofti*, *Brugia malayi*, or *Brugia timori* (1), *W. bancrofti* is responsible for the overwhelming (approximately 115 million) majority. In India, current estimates suggest that 40 million people are infected with *W. bancrofti*, an infection associated with significant morbidity and associated social and economic burdens (2,3).

Among the many clinical manifestations of longstanding infection with the lymphatic filariases [hydrocoele, recurrent adenolymphangitis, lymphoedema or elephantiasis, for a comprehensive review, see (4)], the most intriguing is the asymptomatic (or subclinical) condition associated with high levels of circulating microfilariae (or parasite antigen). Indeed, in areas where *W. bancrofti* or *B. malayi* are endemic, the overwhelming majority of infected individuals have few overt clinical manifestations of their filarial infection despite the presence of circulating microfilariae (and/or parasite antigen) in the peripheral blood. Although they may be clinically asymptomatic, virtually all individuals with *W. bancrofti* or *B. malayi* microfilaraemia have some degree of subclinical disease that includes microscopic haematuria and/or proteinuria (5) dilated (and tortuous) lymphatics when imaged (6,7) and, in men with *W. bancrofti* infection, scrotal lymphangietasia (by ultrasound) (8,9). Despite these findings, the majority of individuals appear to remain clinically asymptomatic for years with only a minority progressing to the acute and chronic stages of infection (1).

The principal pathological changes in chronic lymphatic filariasis result from dysfunction of or inflammatory damage to the lymphatics. Adult worms live in the afferent lymphatics or sinuses of the lymph nodes and induce local changes that result in dilatation of the lymphatics and

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Received: 2 March 2001

Accepted for publication: 11 April 2001

thickening of the vessel walls. Histologically, there is infiltration with plasma cells, eosinophils, and macrophages in and around the infected vessels. There is endothelial and connective tissue proliferation with tortuosity of the lymphatics and damaged or incompetent lymph valves. The overlying skin may show lymphoedema and chronic stasis changes with hard or brawny oedema.

It is believed that some of the pathological consequences of filariasis relate, in part, to the nature of the host immune response to the parasite. In the lymphatics, local immune responses directed toward the adult parasite are believed to cause the granulomatous and proliferative processes that precede total lymphatic obstruction. It is assumed that as long as the worm remains viable, the vessel remains patent (10). Death of the worm, however, leads to local necrosis of a granulomatous reaction around the parasite. Fibrosis occurs and lymphatic obstruction develops; although some collateralization of the lymphatics takes place, lymphatic function remains severely compromised.

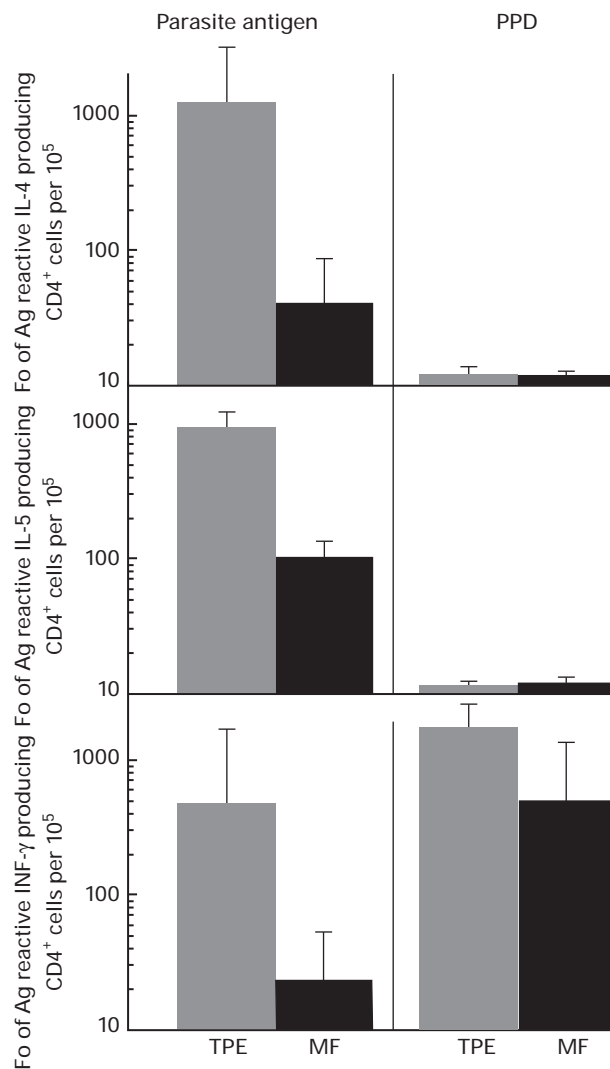
Recent evidence, both from clinical observations (10) and from immunohistological and bacteriological studies of tissue from lymphoedematous limbs of affected patients, has suggested that bacterial or fungal superinfections of limbs with compromised lymphatic function play a significant role in triggering some episodes of adenolymphangitis (11,12) which, themselves, cause or exacerbate the chronic obstructive changes in the lymphatics of affected patients. These studies have shown that the bacterially induced acute processes usually start in the skin and spread along the lymphatics to the lymph nodes.

Delineating the immune responses in lymphatic filariasis – considered to be among the most immunologically complex infections of humans (13,14) – has been a dynamic process with new insights following the identification of each new cytokine, chemokine, cell surface molecule, T cell subset and signal transduction pathway. Still, there has yet to be a theory that unifies the myriad data generated that bear on pathogenesis, protective immunity, parasite-specific unresponsiveness or tolerance. In addition, new diagnostic tools (for *Wuchereria bancrofti* infection at least) have identified ‘active’ or patent infection – the presence of microfilariae and/or adult worms (as assessed by circulating antigen assays, polymerase chain reaction of blood or ultrasonography) – as being the single most important determinant of the nature of the systemic immune response in chronic infection with filarial parasites (15). Despite the superimposition of better clinical definition onto the immunological findings, much interest has continued to focus on the asymptomatic (or subclinical) condition that is associated with high levels of circulating microfilariae (or parasite antigen) and the inability to proliferate or produce interferon (IFN)- $\gamma$  in

response to parasite antigen (16). This lack of responsiveness appears to be directed primarily at the parasite and, in particular, at the stage found in the blood circulation (microfilariae, mf) (17) that represents the major repository of parasite antigen. Whether this antigen-specific hyporesponsiveness is a cause or a result of the heavy intravascular parasite burden remains to be determined.

Similar to most helminth infections, the filariae have complex life cycles that involve several discrete developmental stages within the human host. The immune response to these parasites involves multiple effector pathways directed against different parasite stages, each of which occurs simultaneously within the same host. A useful paradigm by which to understand the immune response to these infections, and the changes that these responses undergo over time, is perhaps to divide the filarial infection into ‘acute’ (or early) and chronic phases. It has proven difficult to focus on acute or early infections in most studies in filarial-endemic regions because the time of infection cannot easily be ascertained. However, ‘acute’ or early infections have been studied using (i): volunteers following experimental infections (18); (ii) temporary residents of or long-term travellers to endemic areas in which the time of initial exposure can be documented (19,20); (iii) young children living in endemic areas (21); (iv) populations that have migrated to an endemic area from a nonendemic area (22); and (v) populations that have become exposed en masse to transmission, such as the military during World War II (23).

The best examples of ‘acute’ filarial infections have been reported in expatriates, deliberately infected volunteers and exposed military forces who, despite relatively short exposure histories, frequently develop clinically apparent allergic reactions (e.g. urticarial rashes) and, in the case of the lymphatic filarial infections, lymphoedema and retrograde adenolymphangitis that is transient. These ‘early’ infections are associated with establishment of parasite specific delayed-type hypersensitivity responses (18), parasite-specific lymphocyte proliferation, cytokine production with a mixed [interleukin (IL)-2, INF $\gamma$ , IL-4 and IL-5] phenotype (24–27), marked eosinophilia and elevated levels of parasite-specific immunoglobulin (Ig)E (19) in the absence of antifilarial IgG4. These findings have been bolstered by the studies in permissive animal models [e.g. jirds (28), cats (29), dogs (30), monkeys (31) and chimpanzees (32)] in which most of these features have been seen early in infection. With the onset of patency (development of adult worms and/or appearance of microfilariae in the blood), certain immune responses are profoundly altered. Most notably, there is a diminution of parasite-specific lymphocyte proliferation, IL-2 and INF $\gamma$  production, an increase in antifilarial IgG4 and the



**Figure 1** Tropical pulmonary eosinophilia (TPE) is associated with an expansion of IL-4-, IL-5 and INF $\gamma$ -producing CD4<sup>+</sup> cells. Geometric mean (+95% confidence limit) of the frequency (Fo) of antigen-reactive CD4<sup>+</sup> cells in response to crude filarial antigen (BmA) or PPD in patients with TPE or subclinical microfilaraemia (mf).

production of the regulatory cytokine IL-10 [and perhaps tumour growth factor (TGF)- $\beta$ ] (26,33–36). The development of this downregulated immune response is determined not only by the duration of infection, but also by parasite burden (number of adult worms and microfilariae), both of which can be a function of the intensity of transmission. In areas of intense transmission, infections are acquired at younger ages and higher parasite burdens likely occur.

Where this paradigm (early versus late infections) breaks down, however, is in acute tropical pulmonary eosinophilia (TPE), a syndrome characterized by an eosinophilic alveolitis and marked elevations of serum IgE and circulating

eosinophils, but in which the majority of patients have evidence of active infection [adult worms on ultrasound (37) or circulating antigen (CAg) positivity]. Whereas microfilariae normally circulate in the blood of patients with lymphatic filariasis without significant clinical consequences, in TPE, microfilariae appear to be trapped in the lung on their first pass through the circulation where they are presumed to initiate an inflammatory cascade. The role of microfilariae in the immune response of TPE has been corroborated by lung biopsy (38), and by high levels of filaria-specific IgE and IgG found in TPE patients (39,40). In contrast to the majority of people with lymphatic filariasis who have a downregulated cellular response to the parasites (41) and a restricted pattern of antibody production, patients with TPE mount a robust systemic and localized immune response, characterized by elevation of both polyclonal IgE and filarial-specific IgE and IgG (42) and the expansion of antigen reactive IL-4, IL-5 and INF $\gamma$  producing T cells (Figure 1). Moreover, filarial-specific IgE and IgG antibodies are found in both the serum and broncho-alveolar lavage fluid of TPE patients; however, the lung antibodies recognize a distinct subset of the filarial antigens recognized by the antibodies in the periphery (42) [the most dominant being the parasite-derived  $\gamma$ -glutamyl transpeptidase (43)].

#### SYSTEMIC CELLULAR IMMUNE RESPONSES IN INDIVIDUALS WITH LONGSTANDING LYMPHATIC FILARIASIS

##### Proliferative responses

The one major conclusion that has been reached consistently from *in vitro* studies of cellular responses to filarial antigens (Table 1) is that proliferative responses to parasite antigen in patients with patent (microfilaraemia and/or circulating antigen-positive) infections are absent or severely diminished (17,26,44–64) although the ability to respond to nonparasite antigens or mitogens is retained. This is generally in marked contrast to the relatively higher parasite-antigen specific proliferative responses seen both in presumably antigen-negative patients with chronic lymphatic obstruction (and/or elephantiasis) and in the parasite-exposed but uninfected (so-called endemic normal/putatively immune) individuals (Table 1).

Because many of these studies were carried out prior to the availability of assays for circulating parasite antigen, we have analysed the relationship between parasite antigen-driven proliferation and circulating antigenaemia using data previously collected on cellular studies in South India and stored serum samples taken at the time of study (Figure 2). As seen, the prevalence of circulating antigen in the clinically asymptomatic mf<sup>+</sup> patients was >95%,

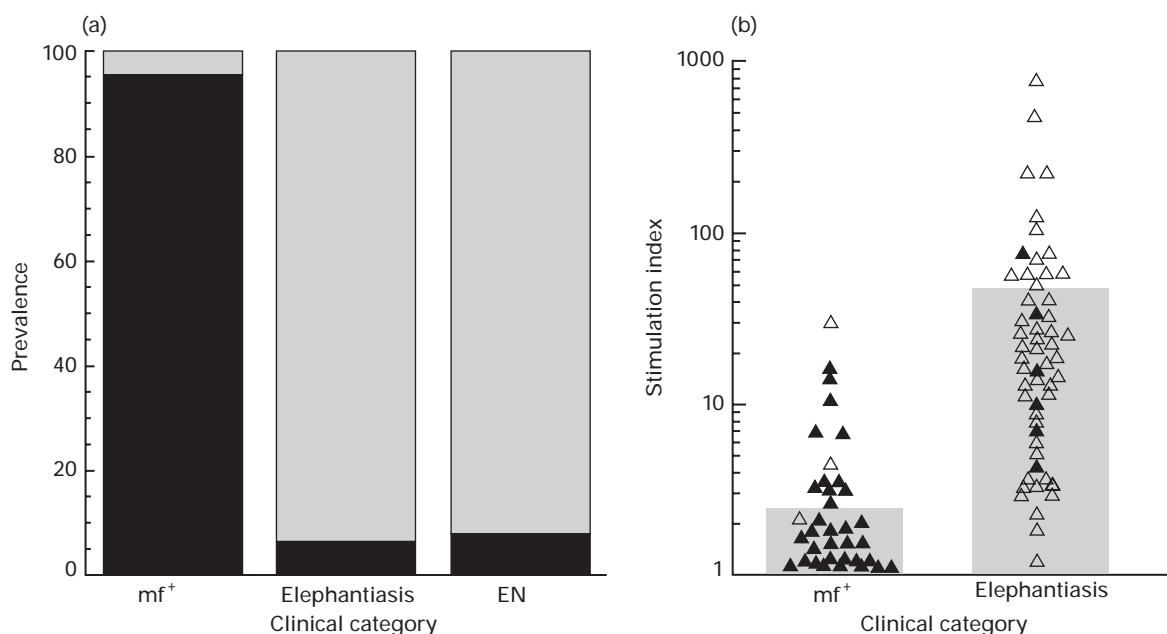
**Table 1** Proliferative responses to filarial parasite antigens and non-parasite antigen(s) in untreated lymphatic filariasis

| Area         | Infectious organism | Antigen   | Diagnostic category <sup>a</sup> |       |                                  |       |                                  |     |                                  |      |         |       | Reference |
|--------------|---------------------|-----------|----------------------------------|-------|----------------------------------|-------|----------------------------------|-----|----------------------------------|------|---------|-------|-----------|
|              |                     |           | mf <sup>+</sup> SC               |       | Dis <sup>+</sup> mf <sup>-</sup> |       | Dis <sup>+</sup> mf <sup>+</sup> |     | mf <sup>-</sup> CAg <sup>+</sup> |      | mf-Dis- |       |           |
|              |                     |           | N                                | SI    | N                                | SI    | N                                | SI  | N                                | SI   | N       | SI    |           |
| Cook Islands | Wb                  | BmA       | 12                               | 1.9   | 10                               | 3.7   |                                  |     |                                  |      | 12      | 4.2   | 44        |
|              |                     | PPD/SK-SD |                                  | ~75   |                                  | ~72   |                                  |     |                                  |      |         |       | 42        |
| Indonesia    | Bm                  | BmA       | 19                               | 1.6   | 15                               | 2.0   |                                  |     |                                  |      |         |       | 45        |
| Indonesia    | Bm                  | BmA       | 9                                | (12)* | 40                               | (66)* |                                  |     |                                  |      |         |       | 46        |
| India        | Wb                  | BmA       | 4                                | 1.2   | 16                               | 2.2   |                                  |     |                                  |      |         |       | 47        |
| India        | Wb                  | BmA       | 10                               | 1.2   | 13                               | 3.5   |                                  |     |                                  |      |         |       | 48        |
|              |                     |           |                                  | 4.0   |                                  | 3.9   |                                  |     |                                  |      |         |       |           |
| India        | Wb                  | BmA       | 7                                | 1.7   | 7                                | 11.5  |                                  |     |                                  |      |         |       | 49        |
|              |                     | PPD       |                                  | 10.1  |                                  | 12.9  |                                  |     |                                  |      |         |       |           |
| India        | Wb                  | WbL3      | 25                               | 3.3   |                                  |       |                                  |     | 25                               | 6.2  |         |       | 50        |
|              |                     | PPD       |                                  | 3.24  |                                  |       |                                  |     |                                  | 3.4  |         |       |           |
| India        | Wb                  | WbmfES    |                                  |       | 7                                | 1.8   |                                  |     |                                  |      | 7       | 2.6   | 51        |
| Haiti        | Wb                  | BpA       | 33                               | 2     |                                  |       |                                  |     |                                  |      | 143     | 2.9   | 52        |
| Haiti        | Wb                  | BpA       | 30                               | 2.4   |                                  |       |                                  |     |                                  |      |         |       | 53        |
|              |                     | PPD       |                                  | 15.1  |                                  |       |                                  |     |                                  |      |         |       |           |
| India        | Wb                  | BmA       | 7                                | 2     | 20                               | 17.8  |                                  |     |                                  |      |         |       | 54        |
|              |                     | SLO       |                                  | 13.6  |                                  | 14.8  |                                  |     |                                  |      |         |       |           |
| Haiti        | Wb                  | BpA       | 11                               | 5     | 22                               | 20-25 | 18                               | 3.8 |                                  |      | 8       | 10.5  | 55        |
|              |                     | PPD       |                                  |       |                                  | ~30   |                                  | ~35 |                                  |      |         | 20-25 |           |
| India        | Wb                  | BmA       | 12                               | 2.2   | 17                               | 48    |                                  |     |                                  |      |         |       | 56        |
|              |                     | PPD       |                                  | 14    |                                  | 34    |                                  |     |                                  |      |         |       |           |
| Indonesia    | Bm                  | BmA       | 43                               | 1.5   | 52                               | 3.2   |                                  |     |                                  |      | 31      | 3.1   | 57        |
|              |                     | PPD       |                                  | 6.1   |                                  | 5.5   |                                  |     |                                  |      |         | 4.4   |           |
| Haiti        | Wb                  | BpA       | 13                               | 8     | 29                               | 40    |                                  |     |                                  |      | 12      | 26    | 58        |
| Haiti        | Wb                  | BpA       | 10                               | 9     | 14                               | 26.9  |                                  |     | 7                                | 12   |         |       | 59        |
|              |                     | PPD       |                                  | 43.6  |                                  | 38.4  |                                  |     |                                  | 68.4 |         |       |           |
| India        | Wb                  | BmA       | 8                                | 1.1   | 11                               | 9     |                                  |     |                                  |      |         |       | 17        |
|              |                     | Bmmf      |                                  | 1.4   |                                  | 10.2  |                                  |     |                                  |      |         |       |           |
|              |                     | BmAM      |                                  | 10    |                                  | 11    |                                  |     |                                  |      |         |       |           |
|              |                     | PPD       |                                  | 21    |                                  | 28    |                                  |     |                                  |      |         |       |           |
| Haiti        | Wb                  | BpA       | 27                               | 6     |                                  |       |                                  |     | 27                               | 9    | 57      | 32    | 26        |
|              |                     | PPD       |                                  |       |                                  |       |                                  |     |                                  |      |         |       |           |
| Cook Islands | Wb                  | BmA       | 20                               | 2     |                                  |       |                                  |     |                                  |      | 19      | 10    | 60        |
|              |                     | Bmmf      |                                  | 1.9   |                                  |       |                                  |     |                                  |      |         | 6.2   |           |
|              |                     | PPD/SLO   |                                  | 62.7  |                                  |       |                                  |     |                                  |      |         | 49.3  |           |
| India        | Wb                  | BmA       | 11                               | 1.7   | 11                               | 4.7   |                                  |     |                                  |      |         |       | 61        |
|              |                     | PPD       |                                  | 45    |                                  | 68.4  |                                  |     |                                  |      |         |       |           |
| India        | Wb                  | BmA       | 12                               | 1.5   | 10                               | 12    |                                  |     |                                  |      | 11      | 13    | 62        |
|              |                     | PPD       |                                  | 14    |                                  | 17    |                                  |     |                                  |      | 18      |       |           |
| India        | Wb                  | BmA       | 8                                | 1.7   | 8                                | 15.8  |                                  |     |                                  |      | 5       | 9     | 63        |
| Indonesia    | Bm                  | BmA       | 11                               | 1.3   |                                  |       |                                  |     |                                  |      | 22      | 1.7   | 64        |

Diagnostic categories include subclinical or asymptomatic microfilariae positive (SCmf<sup>+</sup>), lymphedema and/or hydrocele with or without microfilaremia (Dis<sup>+</sup> mf<sup>+</sup> or Dis<sup>+</sup> mf<sup>-</sup>), those with circulating antigen only (mf<sup>-</sup> CAg<sup>+</sup>) and those without disease, microfilaremia or CAg. SI, Geometric mean Stimulation Index; Wb, *Wuchereria bancrofti*; Bm, *Brugia malayi*; Bp, *Brugia pahangi*. \* % patients responding.

whereas the prevalence of antigenaemia in those with longstanding lymphoedema and elephantiasis was <6%, a finding that reflects not only the widespread availability and use of diethylcarbamazine, but also its known macrofilaricidal activity. In many respects, the immuno-

logical findings in those patients with elephantiasis studied in South India may be considered post-treatment; indeed, inferences about immune mediated pathogenesis in this group may need to be tempered by the fact that relatively few patients with 'chronic pathology'



**Figure 2** Retrospective analysis of the prevalence of circulating antigenaemia (a) and the relationship between parasite-specific proliferative responses and antigenaemia (b) among patients (mf<sup>+</sup> and elephantiasis) and endemic normals (EN) studied immunologically in South India between 1984 and 1998. Dark portion of the bars (a) and the dark symbols (b) represent circulating antigen positivity. In (b), the grey area represents the geometric mean SI of all patients studied.

(17,24,35,36,48,49,54,56,63,65) had active (CAG<sup>+</sup>) infection at the time of immunological study. This fact notwithstanding, insights into the regulation of the immune response in patent *W. bancrofti* infection have been made and will be the focus of the remainder of this review.

Efforts to enhance proliferative responses to filarial antigens by removing potentially inhibitory cell populations have met with little success (46,48,49,66); however, neutralizing antibodies to IL-10 or TGF- $\beta$  [in some (36,56,61,67) but not all studies (68)] have led to modest increases in antigen-driven T cell proliferation; this reversal of the downregulated immune response resulted in proliferative levels that did not reach those seen in 'responsive individuals' without patent infections (i.e. endemic normals or those with chronic lymphatic pathology). Moreover, these inhibited proliferative responses appear to be mostly directed to the microfilarial-containing parasite stages (mf and adult females) (17,69), the stage that is the major repository of parasite antigen.

### Cytokine responses

Direct measurement of cytokine responses to filarial antigens were carried out initially to help explain the immunological differences seen among those with differing clinical manifestations of lymphatic filariasis. Soon thereafter, patterns of cytokine expression were used in an attempt to place the clinical response into the very compelling model of

T cell subset differentiation (Th1/Th2 paradigm) (70). Similar to most host-parasite systems – and particularly one as complex as the human/filarial one – it was difficult to reconcile the model with all of the data collected. In South India, for example, parasite antigen-driven IFN- $\gamma$  and IL-2 (Th1-like cytokines) are downregulated while the Th2-like cytokines IL-4 (almost invariably) and IL-5 (variably) were induced (Table 2). Moreover, IL-10 production could easily be measured in most studies, but this is a cytokine that is produced in humans by both Th1-like and Th2-like CD4<sup>+</sup> cells making the Th1/Th2 paradigm less convincing (14, 16). In contrast, in studies in other regions of the world where lymphatic filariasis is endemic and where these kinds of immunological studies were also performed, IL-5 production in response to antigen was difficult to demonstrate. In addition, the assumption that most of these cytokines were T cell derived needs to be tempered by the findings that, at a quantitative level, the major producers of cytokines such as IL-4, IL-13 and IL-10 may be sources other than T cells (e.g. basophils, eosinophils, monocytes) which can release cytokines when triggered with antigen (71).

### MECHANISMS OF IMMUNE HYPORESPONSIVENESS/ANERGY/TOLERANCE

The long held tenet that chronic, patent filarial infections are associated with antigen-specific immunological

**Table 2** Cytokine expression in studies of lymphatic filariasis in South India in response to various antigens (Ag)

|  | Protein |      |      |      |       | mRNA |      |      |      |       | Fo  |      |      |      |       |
|--|---------|------|------|------|-------|------|------|------|------|-------|-----|------|------|------|-------|
|  | INF     | IL-2 | IL-4 | IL-5 | IL-10 | INF  | IL-2 | IL-4 | IL-5 | IL-10 | INF | IL-2 | IL-4 | IL-5 | IL-10 |
| mf <sup>+</sup> SC                                 |         |      |      |      |       |      |      |      |      |       |     |      |      |      |       |
| BmA  | -       | -    |      | + -  | +     | -    | -    | ++   | ++   | ++    | -   |      | ++   |      |       |
| Bmmf   | -       | -    | +    | + -  | +     |      |      |      |      |       |     |      |      |      |       |
| BmAM   |         | +    | +    | +    | -     | +    |      |      |      |       |     |      |      |      |       |
| PPD/SLO  | +       | +    | -    | -    |       |      |      |      |      |       |     | +    | +    | -    |       |
| Spontaneous  | -       | -    | -    | -    | +     |      |      |      |      |       |     |      |      |      |       |
| mf <sup>-</sup> /CAg <sup>-</sup> Dis <sup>+</sup> |         |      |      |      |       |      |      |      |      |       |     |      |      |      |       |
| BmA  | +       | +    | +    | + -  | +     | -    | -    | ++   | ++   | ++    | -   |      | ++   |      |       |
| Bmmf   | +       | +    | +    | + -  | +     |      |      |      |      |       |     |      |      |      |       |
| BmAM   |         | +    | +    | +    | -     | +    |      |      |      |       |     |      |      |      |       |
| PPD/SLO  |         |      |      |      |       |      |      |      | +    | +     | -   |      |      |      |       |
| Spontaneous  | -       | -    | -    | -    | +     |      |      |      |      |       |     |      |      |      |       |
| mf <sup>-</sup> CAg <sup>-</sup> Dis <sup>-</sup>  |         |      |      |      |       |      |      |      |      |       |     |      |      |      |       |
| BmA  | -       | -    |      | + -  | +     | -    | -    | ++   | ++   | ++    | -   |      | ++   |      |       |
| Bmmf   | -       | -    | +    | + -  | +     |      |      |      |      |       |     |      |      |      |       |
| BmAM   |         | +    | +    | +    | -     | +    |      |      |      |       |     |      |      |      |       |
| PPD/SLO  | +       | +    | -    | -    |       |      |      |      |      |       |     |      | +    | +    | -     |
| Spontaneous  | -       | -    | -    | -    | +     |      |      |      |      |       |     |      |      |      |       |

Qualitative assessment of cytokine responses to parasite and nonparasite antigens based on protein and mRNA as well as frequency of antigen specific cytokine secreting cells (Fo). BmA, *Brugia malaya* adult Ag; Bmmf, *Brugia malayi* mf Ag; BmAm, *Brugia malayi* adult male Ag.

unresponsiveness (or tolerance) has been a convenient way to provide the immunological underpinnings of patent infection, but, as pointed out by others (14,72,73), mf<sup>+</sup> (and/or CAg<sup>+</sup>) patients certainly have the ability to mount particular T cell-dependent antibody responses, and IL-4 production is strong. It appears, therefore, that particular T cell functions are 'tolerized'. This suggests that other regulatory T cells (Tr1/Th3 cells) (74) or dendritic cells (DC2) (75) may actively shape the phenotype of the antigen specific T cell repertoire.

The profound T cell unresponsiveness observed in children of mothers who were microfilaraemic during pregnancy (69,76) can be thought of in terms of neonatal self-tolerance induction. In areas of the world where prevalences reach 80–90%, this may be the major mechanism of filarial-specific tolerance. However, for the regions of the world such as South India where mf carriage is 6–15% and antigenaemia rarely exceeds 20%, other mechanisms of tolerance induction must be assumed. Among the various mechanisms proposed, data generated from studies in South India suggest they may be related to antigen concentration, antigen presentation, induction of apoptosis and suppression.

#### IS TOLERANCE INDUCED BY HIGH ANTIGEN LOADS?

The increased reactivity seen in cellular responses to microfilaricidal chemotherapy of the subclinical microfilaraemic

form of infection with *W. bancrofti* and *B. malayi* has been used as an argument in favour of antigen load being one mechanism underlying the induction of specific anergy (so-called high zone tolerance). In addition, high levels of mf have been shown to lead to the down-regulation of either both Th1- and Th2-induced antibodies (77) or of only the Th1 arm (78). In another study, a positive correlation was found between mf load and IgG4 antibodies (79). Data from jirds infected with *B. malayi* showed a negative correlation between antigen-specific proliferation and mf burden (80) while, in humans, alterations in antigen-driven cytokine production were seen to be related to seasonal fluctuations in mf density (64). This being said, the findings that mf negative adult worm carriers (despite significantly lower antigen loads) have immunological profiles that are similar to those with high levels of circulating mf, suggest that other mechanisms must also play a role in downregulating host immune responsiveness.

Filarial-derived factors have also been isolated that interfere with T cell function. For example, the secretion of immunodominant molecules such as the hapten phosphorylcholine may act as an inhibitor of T cell reactivity (81–84) and, secondarily, as a molecule that diverts the attention of the host immune system. Other molecules with similar inhibitory functions include the *Acanthocheilonema viteae* excretory/secretory (ES) antigen Av17, a

cysteine protease inhibitor which accounts for much of the suppressive activity of filarial ES products on T cell proliferation in murine studies (85). Although their role in immunoregulation is unproven, cytokine-like molecules of filarial origin, including homologues of TGF- $\beta$  (86) and

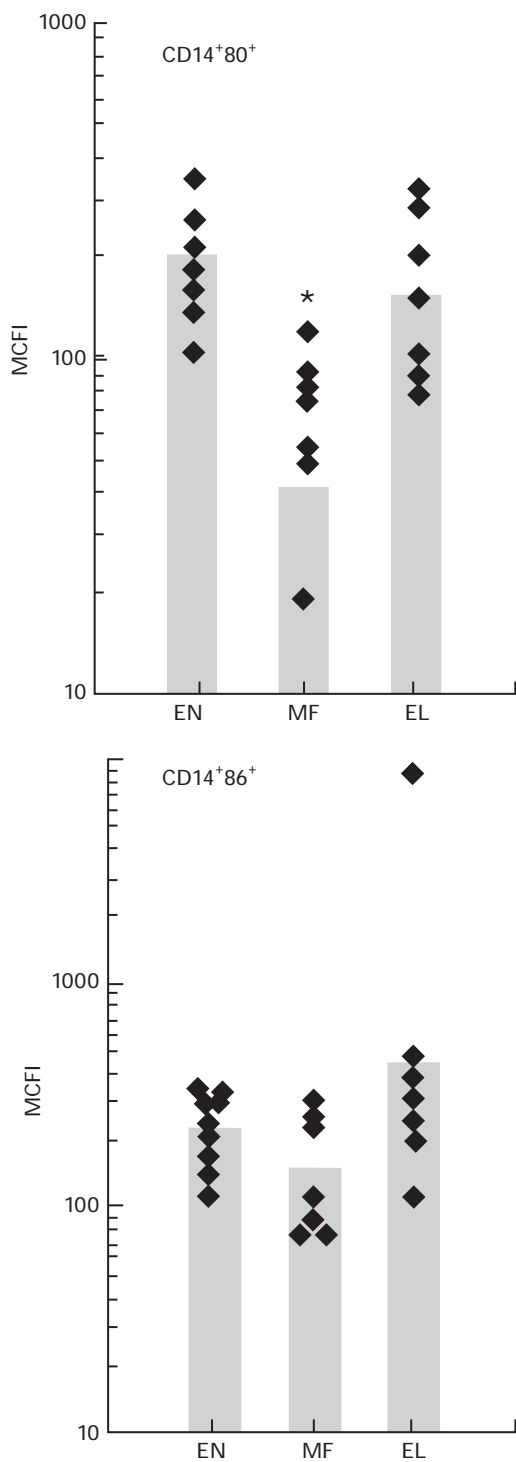
macrophage migration inhibitory factor (87) have also been identified.

### APOPTOSIS

Activation induced or programmed cell death leading to deletion of microfilarial exposed T cells is another mechanism that has been proposed to explain the lack of proliferation seen in patently infected individuals. Indeed, cells that survive activation-induced cell death produce high levels of IL-4 and IL-10 and suppress the proliferation of other antigen-specific T cells, suggesting a mechanism that could prolong *in vivo* tolerance. Apoptotic pathways have not been looked at in detail in human filarial infection. Based on annexin staining, mf<sup>+</sup> individuals from South India do not have increased numbers of annexin positive CD4<sup>+</sup> cells following *in vitro* exposure to filarial antigen compared to appropriate controls (R. Gopinath, unpublished data). *In vitro*, however, high dose microfilarial antigen, if present when CD4<sup>+</sup> CD45RA cells are primed, induces both loss of viability and increased annexin positivity on reexposure to parasite antigen (C. Steel, unpublished data). Furthermore, in mice exposed to *B. pahangi* microfilariae, there have been reported increased numbers of apoptotic cells (J. Osborne and E. Devaney, unpublished data). Such findings warrant further investigation.

### ALTERED ANTIGEN PRESENTATION

The finding that mf<sup>+</sup> individuals have monocytes producing large amounts of IL-10 spontaneously *ex vivo* (36) and data suggesting that mf Ag-exposed dendritic cells function less well in presenting antigen to CD4<sup>+</sup> T cells (88) suggests that the absence of signals normally delivered by antigen presenting cells (APC) costimulatory molecules may induce tolerance. Interestingly, peripheral blood mononuclear cells (PBMCs) from mf<sup>+</sup> patients were found to express low levels of CD80 mRNA compared to exposed by uninfected individuals (63). Although in a study using *B. malayi* infected individuals, exogenous anti-CD28 antibody failed to restore proliferation among anergic T cells from patients (68), flow cytometric data from freshly isolated unstimulated PBMCs from mf<sup>+</sup> South Indians'



**Figure 3** Microfilaraemia is associated with diminished expression of CD80 (top) or CD86 (bottom) on monocytes. Geometric mean channel fluorescence intensity (MCFI) on CD14<sup>+</sup> cells in patients with microfilaraemia (MF) or elephantiasis (EL) in comparison to endemic normal control individuals (EN). Each dot represents a single patient; the shaded columns represent the geometric mean for each group. \*Statistically significant ( $P < 0.001$ ) from other groups.

demonstrated diminished expression of CD80 on CD14<sup>+</sup> cells (Figure 3). Whether incomplete antigen presentation reflects diminished costimulation by CD80 or by 'alternatively activated' APC, as demonstrated in murine systems (89), awaits clarification.

#### CROSS REGULATION BY CYTOKINES AND/OR REGULATORY CELLS

IL-10 and TGF- $\beta$  have emerged as major regulators of T cell responses. Heightened IL-10 responses in patent lymphatic filarial infections have been demonstrated; in these patients, neutralizing anti-IL-10 restores proliferation (35) and enhances type 1 cytokine production by autologous T cells from mf<sup>+</sup> patients (61). Anti-TGF- $\beta$  also enhances the proliferative response, but to a lesser degree than anti-IL-10 (35). Moreover, there was an inverse relationship between IL-10 production and T cell proliferation. Similar data from Indonesian patients with *B. malayi* infections failed to demonstrate anti-IL-10 reversibility of proliferation (68) but did raise the level of IFN- $\gamma$  production.

Regulatory T cells (Tr1/Th3), relatively rare cells whose predominant function is to regulate effector T cell function through the production of the anti-inflammatory cytokines IL-10 and TGF- $\beta$ , have been found to play a significant role in modulating the T cell response in humans in both onchocerciasis and intestinal helminth infections (90). These, along with other regulatory cells (91), some of which may be important in the prevention of autoimmunity or transplant rejection, may provide the link between the suppressor cells identified in lymphatic filariasis and our current understanding of tolerance in infectious diseases.

#### CONCLUSIONS

Antigen-specific unresponsiveness, as it relates to systemic helminth infections (and filarial infections specifically), remains a less than well-understood phenomenon. Defining more clearly its biological basis may rely less on the study of particular molecules and more on a 'systems approach' that can only be assessed using the tools developed for genomics and proteomics. Indeed, it is difficult to identify a simple paradigm that explains all of the observed immunological findings in lymphatic filarial infections. With the rapid movement toward community distribution of chemotherapeutic agents for interruption of transmission and new approaches toward morbidity control in lymphatic filariasis, we must realize that having a detailed knowledge of the processes that modulate antigen specific responses in humans will have

implications well beyond the study of filariasis and other chronic infectious diseases.

#### ACKNOWLEDGEMENTS

The authors wish to acknowledge the large number of scientists and clinicians who have made significant contributions to our studies on the immunology lymphatic filariasis in South India including: Drs V.Vijayshekar, S.P.Tripathy, C.Steel, M.Ravichandran, J.Regunathan, U.Raman, R.Parajape, E.Ottesen, R.B.Narayanan, P.R.Narayanan, A.Maya, S.Mahanty, E.Lobos, R.Lal, C.King, P.Kaliraj, K.Jayaraman, L.Hanna, R.Gopinath and D.Freedman. We also would like to thank the many patients who participated in the studies described herein.

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