Review of Literature
**REVIEW OF LITERATURE**

*Brugia malayi* is an important human pathogen and arguably the major experimental model for filarial nematodes of medical and veterinary significance (Ottesen, 1992). *W. bancrofti* and *B. malayi* are the two important parasites which cause lymphatic filariasis in human. *W. bancrofti* alone is responsible for causing 90% cases of LF globally. It has not been possible yet to grow all the three stages of *W. bancrofti* in the laboratory. Therefore, people have used MF isolated from human blood and L3 isolated from mosquitoes that are artificially fed with the MF. In contrast, all the three different stages of *B. malayi* can be maintained in the laboratory. Therefore, antigenic material for biochemical and immunological studies can be obtained in sufficient quantity. This makes *B. malayi* the choice organism for the LF research and the WHO-sponsored Filarial Genome Project (Williams 1999; Williams et al. 2000) has selected it as the reference organism.

**ANTIGENS:** Filarial nematodes are among the most complex pathogens to invade the mammalian body, and the immune responses to these worms appear correspondingly intricate (Ottesen 1992, Kazura et al. 1993, Maizels et al. 1995). 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 been conducted using crude parasite Ag preparations that contain multiple B and T cell epitopes. Despite this, crude filarial Ag preparation have been shown to be capable of inducing IgE and IgG4 Ab secretion *in vitro* at extremely low concentrations (Nutman et al 1985a, Nutman et al 1985b, Nutman et al 1987). The Ag-induced responses appear to be reciprocally dependent on the frequencies of IL-4 and IFN-γ secreting cell subsets (King and Nutman 1993). The human immune response to filarial infection exhibits many unusual features such as T lymphocyte unresponsiveness, skewed cytokine secretion, and elevated IgG4 production. While these responses are considered filarial antigen-specific, little is known about the spectrum of proteins and other macromolecules, which constitute the target of specific T cells and antibodies. The rapid progress being made in gene discovery and expression of filarial recombinant proteins...
makes it imperative to define the major antigens in natural exposure, and to relate recognition of these antigens to the outcome of infection with filarial parasites. Human antibody response to filarial parasites tends to be dominated by the IgG4 subclass, the IgG isotype least abundant in normal serum Ottesen et al. (1985, Kwam-Lim, Forsyth & Maizels 1990). It is known that the isotype balance is greatly influenced by the nature of infection and disease condition. In filariasis, different clinical categories of infection differ significantly in distribution of antibody isotypes (Hussain, Grogl and Ottesen 1987; Kurniawan et al. 1993).

The four subclasses of IgG are distinct in structure, function, and degree of participation in the antibody response to complex antigens. Hussain et al. (1987) studied both quantitatively (by ELISA) and quantitatively (by immunoblot) differential IgG antibody subclass recognition by BmA soluble Ag in the bancroftian filarial sera. In immunoblot analysis striking associations were seen between IgG4 antibody responses and the asymptomatic state of filarial infection and between IgG3 (and, to a lesser extent, IgG1) responses and the occurrence of lymphatic pathology. Chronic pathology patients showed strong binding to antigens in the higher mol wt region (>68,000) with IgG1, IgG2, and IgG3 subclass antibody and little or no binding with IgG4 subclass antibody. The ASM group, on the other hand, showed strong binding with IgG4 and IgG2, and, to a lesser extent, IgG1. For IgG3, very little binding was observed with ASM sera, although intense binding was seen with CH sera. Quantitatively, the most significant differences among patients’ groups were in the levels of Ag-specific IgG4 antibodies which were more than 17 times higher in ASM patients than those with chronic patients. Thus, the degree and character of immune response made by the host may be the critical determinants of whether (immuno-) pathology develops in association with filarial infections. IgG4 has been shown to possess certain unique features such as functional monovalency (Aalberse et al. 1983), one consequence of which would be the formation of small immune complexes. IgG4 may decrease the complement-fixing ability of IgG1 antibody. The presence of IgG4 antibody may be important in suppressing the development of immunopathology by several mechanisms, especially the helminth infections, and this IgG subclass response may be critical for establishing successful host-parasite relationships (Hussain et al. 1987).
Hussain et al. (1986) also analyzed the IgE responses in human filariasis and observed the parallel antigen recognition by IgE and IgG4 subclass antibodies by immunoblot. Their results clearly demonstrated that IgG4 was primarily responsible for the "parallel" recognition that was seen previously between IgG and IgE antibodies (Hussain and Ottesen 1985). IgG4 may play an important role in modulating IgE-mediated allergic responses in vivo. These findings strongly suggested that IgG4 antibodies might be responsible for the "blocking" effect of IgG antibodies on the IgE-mediated immediate hypersensitivity responses to parasite antigen in patients with filariasis.

Binding patterns of IgG1, IgG2, IgG3, IgG4, and IgE antibodies to adult filarial antigen separated by SDS-PAGE and blotted to nitrocellulose paper were studied in 24 individual sera, eight from each of the three clinical filariasis groups. All sera were tested at a dilution of 1/20. Only IgG subclass consistently showing appreciates similarity to the IgE binding patterns was IgG4.

For IgG1 and IgG2, the binding was predominantly directed toward the higher mol wt components. IgG3 showed the least binding of any of the four IgG subclasses. IgG subclasses have been shown to differ not only structurally and quantitatively, but also functionally, with differences for example in their complement fixing ability, binding to cell membranes, response to different types of antigens, and appearance in different clinical situations (Hussain and Ottesen 1986). Most striking was the observation of similarities between the binding patterns of IgE antibodies and those of IgG4, as distinct from those of the other three IgG subclasses. The predominant binding of IgG1 and IgG2 was with higher mol. wt. antigens. These antibodies often showed less discrete and more diffuse binding. Such binding is frequently associated with the presence of antibody to carbohydrate moieties, and it is well known that carbohydrates evoke a predominantly IgG2 response. Interestingly, MF patients showed intense binding with IgG4 than IgE when compared with those with TPE in which both IgE and IgG4 binding was equally intense. Because the latter is the only group that shows allergic symptoms, it was speculated that IgG4 could be a "blocking antibody," and the ratio of IgE to IgG4 is critical in determining the expression of allergic symptoms.

IgG isotypes differ substantially in their interaction with cell bound Fc receptors (van de Winkel & Capel 1993), and in humans IgGI and IgG3 have the highest affinity for...
the three classes of FcyR (I, II and III) expressed on monocyte and gametocyte surface membranes. IgG4 is relatively ineffective at mediating Fc-mediating cell activation. This relationship may be important in view of the evidence that antibody-dependent cell-mediated cytotoxicity is involved in killing both adult worms and microfilariae anti-L3 response appears to be regulated independently of the response to adult/MF stages, consistent with the hypothesis of concomitant immunity infection-free individuals may share with infected persons an effective protective immunity against new infection. L3 surface antigens are not processed to elicit, or are not of a structural nature which favors, IgG4 production studies in IL-4 deficient mice suggest that Th2 cells are not required to eliminate infective larvae IgG4, a Th-2 dependent isotype, from the antibodies binding to the L3 surface further suggests that Th2 cells may not be essential to protective immunity in filariasis.

Kurniawan et al. (1998a) analyzed the levels of filarial-specific IgG isotypes against somatic extracts of adult, infective larvae (L3) and microfilariae (MF) stages of B.malayi in a brugian filariasis endemic population by ELISA. The major antibody subclasses to each antigen preparation were IgG1 and IgG4, with highest levels IgG4 to adult and MF antigens. IgG1 and IgG4 more equally represented antibodies reactive to somatic extracts of infective larvae. Binding to surface exposed epitopes in immunofluorescence on larval stages is mediated foremost by IgG1 and IgM, secondarily by IgG2 and IgG3 and very little by IgG4. These results indicated that despite extensive common 'house keeping' antigens expressed by all stages, L3 are sufficiently distinct for marked differences in isotype responses to be apparent at the level of whole somatic extracts (Kurniawan et al.1998).

Population from an area endemic for Brugia malayi lymphatic filariasis has been studied for humoral immune responses to filarial parasites (Kurniawan et al.1993). With adult Ag extract, antibody levels of each of the Ig subclasses, IgM and IgE were studied. In ASM individuals, the dominant isotype of antifilarial antibody was IgG4 (88% of total IgG) where as in CH individuals, mostly MF negative, showed substantially higher level of IgG1, IgG2, IgG3 and very low level of specific IgG4. Specific IgE was average 4.5 times higher than ASM individuals.
Yazdanbakhsh *et al.* (1995) in a large study with sera from 146 individuals from a Brugian filariasis endemic area, studied profiles of isotypes of antibodies to two recombinant proteins of *Brugia* sp: Bpa-26: C-terminal portion of the filarial hsp70, representative of a cytoplasmic protein; Bpl-4: A single unit of the tandem repeats of a *Brugia* polypeptide (gp15/400), a secreted product, which is prominently exposed to the immune system. Patients with elephantiasis, who develop chronic obstructive disease, showed high-level antigen-specific antibody responses of isotypes IgG1, IgG2, IgG3, and IgE, while IgG4 levels were dichotomous, high in individuals harboring active infection and low in those who seemed to have eliminated their worm burden (Kurniawan *et al.* 1993, Kwam-Lim *et al.* 1990, Lammie *et al.* 1993). Evidences indicate that induction of IgE synthesis requires two signals: one delivered by the cytokines IL-4 and IL-13, and one delivered by a B cell activating factor. This latter 'second' signal is either delivered directly by T cells (through "cognate interactions") or by T cell-independent factors such as EBV and hydrocortisone (Vercelli 1993). Normal human B cells can generally undergo (polyclonal) μ □ - ε switch upon IL-4 stimulation in the presence of either T cells, T cell membranes, or surrogate ligand for B cell molecules that are engaged during B cell activation such as CD40L (de Vries *et al.* 1993, Aversa *et al.* 1994, Splawski and Lipsky 1994, Gaauchat *et al.* 1994). Activated human B cells may undergo a μ □ - γ4 - ε switch *in vivo* in response to any relevant allergen or to helminth.

Filarial infection is characterized by an immune response associated with the production of Ag-specific IgG4 and IgE and IL-4 an IL-5. Although it is generally thought that a relatively stable relationship exists between the so-called Th1 and Th2 CD4+ human T cell populations, in part through the production of counter regulatory cytokines that suppress the function and growth of the opposite cellular subset (Seder and Paul 1994), CD4+ subset reorientation or bias by Ags occur in certain pathogenic situations (Romagnani 1994). It is established that chronic helminth infections bias CD4+ subset development toward Th2-type responses, which may be causally linked to the immediate hypersensitivity responses associated with these infections (King and Nutman 1992, Wilson 1993, Modlin and Nutman 1993). Tissue-invasive helminth infections are commonly associated with high serum levels of polyclonal and Ag-specific IgG4 and IgE Abs, responses felt to be mediated by the Th2-type cytokines IL-4 and IL-13, and,
perhaps, by the absence of the Th1-type cytokine IFN-γ (Maizels et al. 1993, Locksley 1994).

Garraud et al. (1995) examined the role that parasite Ags themselves play in determining the specific B cell and T cell responses in helminth infections by screening a large panel of recombinant parasite Ags. They attempted to identify the Ags recognized by IgE and IgG4 Abs from patients' sera, obtained from untreated L. loa and O. volvulus infected individuals, using Ag-specific ELISAs. The recombinant filarial proteins were then utilized in an in vitro system to examine the induction of IgG4 and IgE Abs as well as the cytokines responsible for these antibody responses. Several recombinant filarial Ags containing major B cell and T cell epitopes could be identified. These Ags directed IgG4 and IgE antibody secretion both in vivo and in vitro in filaria-infected patients in an IL-4 and an IL-13-dependent fashion.

IL-12 has been shown to down-regulate IgE production indirectly by promoting the expression of Th-1 cells and the secretion of IFN-γ and by inhibiting the development of IL-4 producing cells (Manetti et al. 1993, Trinchieri 1993). IL-12 also acts directly by regulating the CD28/B7 Ag interaction on the surface of T and B cells (Kuhn et al. 1994) and by suppressing the synthesis of IL-4 induced IgE (Kinawa 1992). IL-12 has also been shown to induce IFN-γ transcription and protein synthesis but not to down-regulate IL-4 transcription and synthesis by allergen-specific human Th2 clones (Yssel et al. 1994). This suggests that IL-12 act by inducing Th2 cells to switch to a Th0-type rather than to a Th1-type profile of cytokine secretion.

Dimock et al. (1996) have reported that Th1-like antifilarial immune responses predominate in antigen-negative persons. The absence of circulating filarial antigen was associated with Th1-like responses, including significantly higher proliferate (p<0.001) and IL-2 (p=0.008) responses and a higher prevalence of gamma interferon, the elevated Th1-like responses in antigen-and microfilariae-negative individuals are consistent with the hypothesis that these responses contribute to protection in putatively immune individuals. Proliferation in response to mitogen (PHA) and nonparasite antigen (PPD) was not significantly different among the three categories of filarial patients. Individuals differ in responsiveness to adult filarial antigens, based on microfilariae status, but these studies did not distinguish between antigen-negative and antigen-positive individuals. We have
extended these observations by determining that increased proliferate responses to adult antigen are associated with the absence of circulating filarial antigen. Taken together, these results indicated that in the absence of circulating filarial antigens, Th1-like responses dominate (Dimock et al. 1995).

Kurniawan et al. (1998b) reported the specificity of predominant IgG4 antibodies to adult and microfilarial stages of *Brugia malayi*. In preliminary studies with sera samples collected from 120 subjects from brugian filariasis endemic area, only IgG1 and IgG4 isotype showed strong reactivities among most subjects; IgG3 responses were poor and no IgG2 reactions could be detected. The only antigen associated exclusively with microfilariae was a diffused 38-kDa component. Overall preferential reactivity of IgG4 antibodies to low mol. wt. components show that IgG4 dominates in the microfilaraemics, as well as in those endemic normal who are seropositive, while elephantiasis patients show a higher level of IgG1 reactivity.

de Boer et al. (1997) reported that IL-12 suppresses IgE production, but enhances IgG4 production by human PBMCs. IgG4 and IgE isotypes contribute marginally to the pool of circulating antibodies in healthy individuals, but are elevated during atopic diseases and particularly upon helminth infections. Studies by de Boer et al. (1998) showed that IgG4 produced by PBMC of filarial patients in vitro correlates strongly with plasma levels of high IgG4 levels, but such correlation was not found for IgE. There results also revealed that in individuals with elevated IgG4, the B cell compartment in PBMC carries cells that are already committed to IgG4 production and are independent of Th2 cytokines, IL-4 and IL-13. However, IgE release by PBMC was found to be dependent on IL-4 and IL-13.

**EXCRETORY-SECRETORY-ANTIGENS**: Parasite nematode excretory-secretory (ES) products consist of an ill-defined collection of molecules released by the parasite that have postulated roles in nutrition, migration, reproduction, immune evasion and molting, in addition to waste products (Maizels et al. 1988, Hotez et al. 1995; Lightowlers et al. 1988; Chenthamarakshan et al. 1996; Frank et al. 1999). The general method of collecting and studying ES material has been to culture nematode larvae or adult worms in vitro for sufficient time to allow its accumulation in the media. In
addition to the above process, research on ES products has been conducted to identify diagnostic antigens (Kaushal et al. 1984; Dikshit et al. 1995) and vaccine candidates (Lucius et al. 1991, Frank et al. 1999).

**ANIMAL STUDIES**

Li et al. (1993) reported that vaccination of jirds with recombinant filarial paramyosin (BM5-MBP-fusion protein of *B. malayi* paramyosin and maltose-binding protein) induced significant protective immunity; adult worm recoveries, worm lengths, and blood MF counts were reduced. Interestingly, protective immunity was not induced by immunization with *Dirofilaria immitis* paramyosin. Sera from the immunized animals were only reactive with native paramyosin at 97kd in immunoblots performed with *B. malayi* adult worm PBS extract.

Lawrence et al. (1994) reported that adult and microfilarial stages of *B. malayi* stimulate contrasting cytokines and Ig isotype responses in BALB/c mice. The adult parasites, and females in particular, exerted a rapid polarization of the immune response and spleenocytes taken from infected animals secreted high levels of IL-4 in a specific response to filarial Ag. The IL-4 secretion is principally by CD4+ cells and can thus be attributed to the activation of Th2 cells. This selective response was also reflected in the Ig isotypes expressed, with enhanced total IgE and specific Abs present of only IgM and IgG1 (which are promoted by IL-4). It seems that in adult worm-infected mice, secreted IL-4 (and perhaps IL-10) blocks the class switch to IgG2a, IgG2b, and IgG3 (Snapper and Paul 1987).

The response to MF at the cytokine level was very different from that to adult worms. An initial strong IFN-γ response to MF was accompanied by a later modest Th2 response. IgG1, IgG2a, IgG2b, and IgG3 responses to filarial Ags were highly elevated, but there was no increase in total IgE. These results are similar to those of Pearlman et al. (1993) who reported an early peak of IFN-γ response to *B. malayi* in BALB/c mice.

The individual kinetics of of the IL-4 response to MF (delayed) and to adult females (immediate) indicates that they are differentially regulated. Possible explanations of the later IL-4 response to MF is that IL-4 is induced by large or chronic Ag doses that exceed a critical threshold point with time (day 28 p.i), however, even at
doses as high as 10^6 MF, no immediate IL-4 response was induced. The possibility that chronicity of exposure to Ags leads to Th2 response remains to be tested.

Mice receiving adult female worms showed a dominant Th2 response despite the continual release of MF. Evidently, the females exert a strong enough influence on the immune system to override the Th1-type response caused by the MF themselves. A further contrast was seen between female and male worm recipients in that the IL-4 response to adult males was much weaker.

These results imply that female worms possess or secrete specific molecular components that augment the bias towards Th2 responses. These components are most likely to be derived from the uterus and to be involved in egg formation and MF release. Contrastingly, in *Schistosoma mansoni* infection, the egg stage, and not the adult worm, is a potent inhibitor of Th2 responses (Pearce and Sher 1991, Pearce et al. 1991); and both the larval (Bancroft et al. 1993) and adult filariae consistently stimulate Th2-type responses, whereas late stage schistosomes do not (Pearce and Sher 1991).

A delicate balance maintained in the host between the cellular response to adult female worms and MF may be responsible for the differential spectrum of disease manifestation seen in human filariasis. Adult worms stimulate a very strong Th2 response, yet individuals with lymphatic filariasis display little evidence of an allergic response, and many ASM individuals show no adverse signs at all. In this context, MF-driven IFN-γ may modulate a potentially pathogenic Th2 response in the host by opposing some of the effects of IL-4. This is in accord with recent reports that IgE levels are reduced in MF-positive patients, although IgG4 responses are enhanced (Kurniawan et al. 1993) The class switch to both IgE and IgG4 is promoted by IL-4, but IgE is more sensitive to down-regulation by IFN-γ than IL-4 (Ishizaka et al. 1990).

More striking effects of microfilaremia are seen in animal models. In *B. pahangi*-infected cats, the presence of filarial-specific IgE correlates with the death of adult worms and most cats with high levels of circulating MF do not produce IgE (Baldwin et al. 1993). Investigation of the ability of ES-products of MF or adult female worms (Miller et al. 1991) to drive immune responses in a Th1 or Th2 direction may elucidate how adult worms are capable of polarizing the immune response.
Lawrence et al. (1995) reported that infection of IL-4-deficient mice with *B. malayi* demonstrated that host resistance is not dependent on a Th2-dominated immune response. In wild-type mice adult female worms induce Th2 responses, characterized by antigen-specific IgG1 production, elevated IgE, and marked IL-4 secretion by spleenocytes stimulated in vitro with Brugia extract. However, L1 (MF) induce Th1 responses with the appearance of antigen-specific IgG2a, IgG2b, and IgG3 and IFN-γ secretion by spleenocytes.

Infection of IL-4 deficient mice revealed a dramatic change in the response to adult worms, with a severe reduction in IgG1 (human equivalent of IgG4) production and a corresponding increase in the production of IgG2a, IgG2b, IgG3 isotype antibodies, and IFN-γ release. This switch to Th1-type responses was particularly marked in IL-4-deficient recipients of female worms, which continually release live MF. In the absence of IL-4, down-regulation of the MF-induced Th1 response does not occur. Despite these profound alterations to the immune response in IL-4-deficient mice, survival of L3, adult worms, or MF in the peritoneal cavity was unaffected. In mice, therefore, the prominent Th2-type response elicited by filarial parasites may not be an essential component of the host protective immune response (Lawrence et al. 1995).

Adult female filarial worms, which survive for many years in infected humans, induce a strong Th2 response. In contrast, exposure to MF alone induces a primary Th1 response. Therefore the female worms, which are constantly producing the *in vivo*, apparently override the Th1 response to MF derived from the females. Adult female worms induce a rapid Th2 polarization of the immune response when implanted into BALB/c mice; while MF, in the absence of adults, stimulate Th1 cells (Lawrence et al. 1994). The Th1 response in the IL-4-deficient mice to adult female worms is probably not a reaction to the female adults, but to the MF that they are continually releasing, as adult male worms (and L3) stimulate little IFN-γ even in the IL-4 knockout animals. In wild-type animals, the dramatic IL-4 response to the female worm precedes and overrides any Th1 response that might be mounted to MF. The female *Brugia* parasite can therefore be seen as the major player manipulating the immune system in this complex parasitic disease. The parasitic clearance is not, at any stage of infection, dependent on IL-4 induced Th2 responses.
Pearlman et al. (1995) examined the IL-12 modulation of T helper responses to *Brugia malayi*. IL-12, a heterodimeric cytokine produced by B cells and macrophages, is a potent inducer of NK and T cell IFN-\(\gamma\) production (Chan et al. 1991, German et al. 1993; Mountford and Pearlman 1998), and favors development and activation of Th1 cells. They observed that IL-12 treatment suppressed induction of filarial driven Th2 responses and modulated recall responses of established Th2 cells, but did not alter elimination of blood-borne MF in nonimmune or immune mice. Development of a Th1 response and production of IFN-\(\gamma\) are required for control of murine infection with *Leishmania major* (Heinz et al. 1989; Sadick et al. 1990), whereas activation of the Th2 subset and production of IL-4 or IL-5 are essential for elimination or limiting migration of the intestinal nematodes *Heligmosomodes polygyrus* (Urban et al. 1991), *Nippostrongylus brasiliensis* (Urban et al. 1992), *Trichuris muris* (Else and Grencio 1991; Else et al. 1994), and *Strongyloides venezuelensis*. Mice treated with rIL-12 are protected from infection with *Listeria Monocytogenes* (Heish et al. 1993), *Toxoplasma gondii* (Gazzinelli et al. 1993), or *L. major* (Heinz et al. 1993).

Acquired resistance to *B. malayi* MF is associated with selective induction of a Th2 response (Pearlman et al. 1993). It is not known whether IL-12 preferentially stimulates Th 0 cells to develop into IFN-\(\gamma\)-producing Th1 cells or stimulates mature Th1 cells to secrete higher levels of IFN-\(\gamma\).

It is also possible that IL-12 acts directly on filarial specific CD4\(^+\) Th2 cells to enhance production of IFN-\(\gamma\), a possibility supported by the observation that addition of IL-12 to human Th2 clones induces transient production of low levels of IFN-\(\gamma\). In any case, we found that the effect of IL-12 was dependent on endogenous IFN-\(\gamma\), because neutralization of this cytokine in vivo completely reversed IL-12-induced inhibition of IL-4 and IL-5 production and eosinophil recovery. It is possible but Th2 cells are not absolutely required for elimination of MF and that other mechanisms such as Ab (Kazura et al. 1982) may be important.

Babu et al. (2000) investigated the role of IFN-\(\gamma\) and IL-4 in host defense against *Brugia malayi* in the mouse model, using SCID and IL-4 knock out mice. Their study suggested that both IL-4 and IFN-\(\gamma\) play some role in host resistance to *B. malayi*. The
complete clearance of *B. malayi* is delayed in the absence of either pathway. The effects of the lack of IL-4 are more profound in that patent infection develops in its absence.

**CYTOKINES AND FREQUENCY OF DIFFERENT CYTOKINE PRODUCING CELLS**

de Almeida *et al.* (1998) investigated the frequency of cytokine-producing cells in antigenemic and nonantigenemic individuals with *Bancroftian filariasis*. Both unfractionated PBMC and purified CD4 T cells were analyzed by Flow Cytometry for intracellular cytokine production. Intracellular cytokine staining of mitogen-stimulated PBMC showed that the frequency of either IFN-γ - or IL-4-producing cells was higher in the nonantigenaemic individuals with clinical filariasis (CFA negative CH individuals) than in the ASM.

Both ASM and CFA positive CH individuals i.e. actively infected individuals have frequency of IFN-γ-producing cells lower than in the CFA negative CH patients. Frequency of IL-4-producing cells in actively infected individuals was lower than in the CFA negative CH patients. No difference in the frequency of IFN-γ, IL-4, IL-5-producing cells in purified CD4^+^ T lymphocytes were found among the groups. These findings suggested that the presence of antigenaemia, which is an indicator of current active infection, is closely associated with the frequency of IFN-γ and IL-4-producing cells in lymphatic filariasis. Cytokine responses were closely associated with the presence or absence of active infection, and a role for non-CD4^+^ cells in cytokine production by filariasis patients is suggested. Individuals, who have CFA, whether they are ASM or CH, seem to have a diminished capacity to produce parasite-specific IFN-γ compared to CFA negative CH patients. No correlation was found between cell frequency and supernatant levels of any cytokine examined in the unfractionated PBMC. This lack of correlation, along with the similar frequencies of cytokine-producing cells between patients groups in purified CD4^+^ T cells, suggests that a subset of cells which are non-CD4^+^ T cells are involved in the differences of cytokine expression and levels of secretion among patient groups.

CD8^+^ T cells are good candidates to be important sources of cytokine in lymphatic filariasis. CD8^+^ T cell infiltration in the tissue biopsies from CH individuals has been reported (Freedman and Berry 1992). CD8^+^ T cells can be a major source of IL-5 production in patients who have cleared infection (de Almeida *et al.* 1996). Persons with
clinical pathology have elevated levels of soluble CD8\(^+\) molecules and of CD8\(^+\) HLA-DR\(^+\) T cells in their circulation (Lal et al. 1989, 1990).

To understand the co-ordinate expression of the Th1 and Th2 cytokines at a single-cell level, de Boer et al. (1998) stimulated PBMC from filariasis patients (ASM) with B. malayi adult worm antigen and analyzed for co-expression of cytokines by intracellular staining. Their studies indicated that in human peripheral T cells the co-expression of the dominant Th1 and Th2 cytokines within a single cell is a rare event and that IL-13 is more frequently associated with a Th2 than Th1 type response in primary T cell culture.

de Boer et al. (1997) reported that IL-12 suppresses IgE production, but enhances IgG4 production by human PBMCs. It is well known that IgG4 and IgE isotypes contribute marginally to the pool of circulating antibodies in healthy individuals, but are elevated during atopic diseases and particularly upon helminth infections. Studies by de Boer et al. (1998) showed that IgG4 produced by PBMC of filarial patients in vitro correlates strongly with plasma levels of high IgG4 levels, but such correlation was not found for IgE. Their results also revealed that in individuals with elevated IgG4, the B cell compartment in PBMC carries cells that are already committed to IgG4 production and are independent of Th2 cytokines, IL-4 and IL-13. However, IgE release by PBMC was found to be dependent on IL-4 and IL-13.