review of literature

Lymphatic filariasis is a tropical disease, caused by mosquito-borne nematode parasites, which are currently affecting about 120 million people all over the world (90-92). A human host gets infected when of the infective larvae is delivered on the skin by an infected mosquito while taking the blood meal. They get into the lymphatics, where they mature to *dioecious* female and male adult worms. The adults live for about ten years, producing millions of microfilariae (MF). The larval form of the parasite is found in peripheral blood of certain infected individuals. As the filarial worms do not replicate in the human host, they have evolved sophisticated mechanism of immune evasion and modulation for their long-term survival. They survive till they are picked up by a suitable vector to complete their life cycle (93). The nature of these evasive mechanism, and the differences between protective and non-protective immune responses, are the central questions now being addressed in filariasis (94). It has been a difficult thing to formulate a specific protective mechanism against the disease. Filariasis is a debilitating and often disfiguring disease. No definite protection against this disease either humoral or cellular has been formulated. It is often seen that if the disease is detected early, it can be controlled by DEC treatment. Many investigators for assessing lymphocyte responsiveness have collected immunological data in human filariasis.

2.1 Immunological Profile.

Onchocerca spp induce both cellular and humoral immune reactions as in case of lymphatic filariasis. Study on delayed type of hypersensitivity reactions to antigenic extracts of adult *Onchocerca volvulus* show patients with active form (sowdah) or the more common form of low grade Onchocercal Dermatitis.

Bartlett et al (1978) observed that individuals with active dermatitis produce a delayed type of skin rsponse (95). The studies also suggest that cellular

reactions to filarial but not to unrelated antigen are suppressed in individuals with high parasite levels, a situation that is also observed in lymphatic filariasis. On the other hand, a decreased ability of lymphocytes to proliferate *in vitro* after stimulation with mitogens has also been described in Onchocerciasis (96). Filarial worms induce both cellular and humoral immune responses in the infected host, but the exact nature of the antigens that elicit these reactions remain almost entirely unknown. Because filarial parasite undergo marked morphological changes during their development in the vertebrate host, the existence of both unique stage specific antigens and antigens common to different stages of the parasite could be anticipated and is now confirmed to exist. For many years, it has been known that a multitude of antigens are shared not only by different stages of a given filarial parasite but also by filarial worms belonging to different species. It has become clear that distinct developmental stages of filarial worms also posses unique, stage specific antigens, in addition to "public" antigens shared by many stages of the parasites (97). Antigens associated with internal organs performing basic functions essential for survival of the worms are likely to be shared by many parasite stages. They also undergo marked changes during the development of the worms, *e.g.* the external surface, the digestive system and the reproductive organs, are more likely to be stage specific (98). Antibodies reacting with somatic, secreted or surface antigens of filarial worms can be detected by a variety of methods (99-102). Such studies reveal that antifilarial antibodies belonging to IgM, IgG and IgE immunoglobulin classes are elicited during filarial infection. IgE responses to metabolic antigens of microfilaria may contribute to the pathogenesis of the acute symptoms to tropical pulmonary eosinophilia (103). IgM or IgG antibodies directed against surface antigens or microfilaria most likely contribute to the clearance of the parasites from the blood (104-108).

The "immunosuppressed" state has been most clearly been demonstrated *in vitro* studies of lymphocyte function (109, 110). It has been seen that patients with chronic infection either respond poorly or do not respond at all to filarial antigens, but their responses to other antigens and to mitogens remain normal. Direct proof that filarial infection in nature confers resistance to reinfection with the same parasites is almost non-existent. However, evidence for this phenomenon exists in several infected animal models (110). Cotton rats are resistant to reinfection with *L. carinii,* the growth of the larvae in a challenge infection is retarded, their moults are delayed, and the few adult worms that develop are permanently stunted. What mechanism mediates this type of resistance is not known but it appears to occur during the first week of larval development (111-113). No evidence for a similar phenomenon could be obtained in a rhesus monkey infected with *Brugia malayi* (114). Human beings living in an endemic area are likely to be exposed to small numbers of infective larvae over prolonged period (115). Donald et al reported in their paper that lymphatic filariasis is manifested by a spectrum that range from microfilaremia to gross immunopathology. The geographical variation seen in this disease has been explained by heterogeneity in genetically determined host responses. The model shows that there is a sequential progression from infection, microfilaremia, amicrofilaremia to obstructive disease in all individuals who experience microfiaremia. Only the probability of developing microfiaremia is geographically variable, being dependent on the local incidence of infections. Nutman summarised 17 published studies involving human hosts who have been experimentally infected with filarial parasites. Over the past 60 years, 45 individuals have been deliberately infected with *Wuchereria bancrofti, Brugia malayi, Brugia pahangi, Loa loa, Mansonella, Perstans Mansonella, Ozzardi* and or *Onchocerca volvulus.* The finding from these experimental infections have helped to define microfilarial survival and periodicity within human hosts, the

prepatent period of the causative agents of lymphatic filariasis, aetiologic agents for particular clinical syndromes, immunologic and heamatologic consequences of filarial infection and the role of chemotherapeutic agents (116).

2.2 Helminthic Infection & The Immune System with Special Reference to *W.Bancrofti* Infection.

Helminths are a biologically complex-group of parasites multicellular and often macroscopic and/or microscopic at larval stages. Although helminth infection are most often associated with Th-2 mediated immune responses, such as elevated IgE levels and circulating eosinophils, Th-1 mediated responses can be measured and in some cases are associated with protection. As The complexity of the life cycles of helminths provide ample opportunity for stimulation of distinct T-Cell subsets by different parasite stages in different hosts, one such example is murine schistosomiasis, where Th-1 responses are observed during the first several weeks of infection. However, at all time when these worms begin laying eggs there is a dramatic switch in the pattern of cytokines observed in infected animals towards a Th-2 response (117). The induction of Th-2 response appears to be directly related to the presence of egg antigens, since injection of eggs, or a soluble egg antigen preparation into naive mice include a Th-2 like response (118). Interestingly, it appears that both Th-1 and Th-2 cells are responsive to schistosome antigen maintained in schistosome infected mice, since addition of neutralising antibodies directed against IL-10 *in vitro* to spleen cell culture up regulates the production of INF-γ (119).

Another well studied helminth infection, where differential T-Cell responses are associated with the outcome of infection is *Trichinellasis.* It was observed that the enhanced expulsion of these gut dwelling worms, found in

certain mouse strains, is associated with elevated Th-1 responses, while decreased expulsion can be correlated with enhance Th-2 responses (120). An interesting aspect of these studies was that the type of T-cell response was distinct, depending upon the lymphoid organ examined, providing support for the concept that these responses can be compartmentalised (121). It has been suggested that regulatory interactions at the T-cell level contribute to wide range of clinical presentation observed with human filarial infection (122). One of the largest group of filarial patients appear to be those, who have no evidence of disease, but have microfilaria circulating in their blood while another group of patients have been found to exhibit moderate to severe disease. Some individuals in endemic areas have no evidence of infection, but exhibit strong immune responses against parasite antigen. Studies are currently focused on defining the role of T-Cells and Cytokine profile in patients with varying disease patterns.

One of the major problems in the study of immunity to filarial worms and the development of filarial vaccines is non-availability of suitable animal models. Neither *Wuchereria bancrofti* nor *Onchocerca* develop patency in laboratory animals, while patent infection with *Brugia spp* are confined to animals that are immunologically less well defined such as birds, ferrets, dogs and cats (123). Interestingly, the inability of *Brugia* to develop in mice has been directly linked with the immune response since it was shown that the nude mice and the SCID mice could develop a chronic infection (124,125). In the SCID mouse, *Brugia malayi* develops a patent infection and over one-half of the animals exhibit circulating microfilaria (125). Such models may facilitate our understanding of the role of immune response in protection as well as pathology, and SCID-*hu* most can be used in vaccine development. Murine models can be useful for evaluation of immunity to specific life cycle stages, since both larvae and microfilariae can survive in mice for varying degrees of time following

inoculation. Recently this approach has been applied to the development of a vaccine against Onchocerca (126). Its larval stage survives and grows within a micropore chamber after subcutaneous implantation in mice. Localisation within the chamber allows recovering of the larvae, which normally would migrate throughout the skin after inoculation. In both lymphatic filariasis and onchocerciasis, vaccination studies have focused on the infective L3 larvae and microfilarial stages of the life cycle. The feasibility of an anti-infective stage vaccine is supported by experiments in animal models in which resistance to filarial infection is induced by vaccination with attenuated L3 larvae (126,127). Vaccines against microfilariae (MF) are aimed at blocking transmission and in onchocerciasis immunity against this stage of the life cycle has been induced in variety of experimental models with live parasites. Although vaccine-induced immunity against MF of Onchocerca could result in pathology similar to that induced by the antifilarial drug ivermectin, the responses induced must be regulated carefully to avoid the promotion rather than reduction of the disease (127). This should not be a matter of concern, with vaccines directed against L3 larvae (128).

2.3 Cellular and Humoral Immunity.

The specific humoral immune response is represented by B-Cells, whose induction depends on T helper cells cellular immunity is represented mainly by T-Cells that act locally via contact on a one-to-one cell basis in solid tissues. The biologically active products of B-Cells, antibodies multiply the effect unit of one B Cell more than 100,000 times. Both T Cell and antibodies have in common a uniform and stereotyped mechanism, which focuses on to the site where foreign antigens are present.

2.3.1 Cellular Responses.

T-cells recognise antigens only when they are presented in association with self-MHC molecules on the surface of antigen presenting cell. Introduction and triggering of effect mechanisms of T cells are often mediated via their specificity for self-MHC products. Effect function of antibody is mediated via the constant portion, which either binds to Fc receptors (e.g. IgE on mast cells or basophills) or binds to Clq, initiating the complement cascade. Therefore effect functions triggered via T-Cell or antibody molecules are similar; both link specificity for foreign antigenic determinants with stereotyped receptors for mediating effector functions. Studies on cell-mediated immune (CMI) responses have revealed that patent infections were associated with a marked state of unresponsiveness to parasite antigens, while reactions to unrelated antigens remained normal (129-131). This has been demonstrated by *in vitro* proliferative responses of lymphocytes to crude antigenic extract of microfilariae and adult worm (132-134). The hypo-responsiveness appears to result from the activation of immuno-regulating circuits that specifically suppress lymphocyte reactions to parasite antigens. Three mechanisms that regulate these reactions during human infections with *B.malayi* have been identified; adherent suppresser cells, non-adherent suppresser cells (presumed to be thymus derived lymphocytes) and some yet undefined suppressive serum factors. The antigen specific immune un-responsiveness in Bancroftian filariasis was studied by analysing interleukin-2 and interferon gamma production by their sub population taken from patient with various clinical symptoms (135,136).

In one of the study of IL-4, its has been seen by Salerni et al that IL-4 has an essential role in delayed hypersensitivity reaction, as illustrated by contact sensitivity (CS) to trinitrochlorobenzene (TNCB). Injection with monoclonal

antibody to IL-4, but not with control antibody, reduced contact sensitivity after active immunisation by 75%, as measured by ear swelling. The histological alteration of CS was also reduced. IL-4 was essential to the effector stage, as inhibition of its production or action blocked the passive transfer of CS in particular, treatment of immune lymph node cells with antisense-oligonucleotide to IL4 inhibited the systemic transfer of CS. Transfer was also inhibited by monoclonal antibody to IL-4 given to the recipient. These results indicate that IL-4 is an essential cytokine at the effector stage of the contact sensitive CS reaction (137). Local host immune responses to the lymphoid dwelling filarial parasite Wuchereria *bancrofti* are important in the pathogenesis of the lymphangitis that leads to filarial elephantiasis caused by *W.bancrofti* infection and to up-regulate class I MHC expression on human umbilical vein endothelial cells as compared to unstimulated control supernatants from the same individual (relative fluorescence intensity =159% +/- 13.5; P<0.001)(138). In contrast, individuals with the same filarial infections but manifesting no lymphatic disease were unable to generate, in response to filarial Ag the cytokines required for this activation Supernatants induced by non-filarial Ag such as purified protein derivative (PPD) were able to effect class I MHC up regulation in both patient groups. The filarial Ag driven supernatants did not cause detectable class II MHC on human umbilical vein endothelial cells. These results suggested a likely role for parasite Ag driven, cytokine-mediated endothelial cell activation in the pathogenesis of lymphatic inflammatory/obstructive filarial disease.

The immunological mechanism involved in maintenance of asymptomatic microfilaremic state (MF) in patients who have impaired filarial antigen (Ag)-specific lymphocyte proliferation and decreased frequencies (Fo) of Ag-specific-T-cells and yet elevated serum IgE and antifilarial IgG4 titres is not clearly understood. To investigate the mechanism of Ag specific angry in MF carriers in

contrast to amicrofilaremic individuals with chronic lymphatic obstruction (C.P.), the frequency of Ag-specific lymphocytes from peripheral blood mononuclear cells secreting either IL-4 or IFN-γ were assessed by filter spot enzyme-linked immunosorbent assay, and IL-10 and transforming growth factor-β (TFG-β) mRNA transcript levels were measured assessed by semi-qualitative reverse transcriptase polymerase chain reaction. The Fo of Filaria specific IL-4- secreting lymphocytes were equivalent in both MF (geometric mean[Gm]=1:11,700) and cp (gm=1:29,300 p=0.08), whereas the Fo of IFN-γ-secreting lymphocyte were lower in MF (gm=1:39,300) than in Cp (Gm= 1:4200, p>0.01) when the ratio of IL-4/IFN-γ (T helper type 2(Th-2/Th-1) secreting cells were examined, MF subject showed a predominant Th-2 response (8:1) compared with a Th-1 response in Cp individual (1:4) mRNA transcript levels of IL-10 were also significantly elevated in MF compared with Cp individuals (P<0.01). Further, IL-10 and TgF-β were shown to have a role modulating the Ag-specific energy among MF subject, in that neutralising ant IL-10 or anti TGF-β significantly enhanced lymphocyte proliferation response (by 220-1,300%) to filarial Ags in MF individuals. These findings demonstrated that MF subject respond to parasite by producing a set of suppressive cytokines that may facilitate persistence of the parasite within humans while producing little clinical symptoms of the disease (139). The persistence of microfilaria in the blood or skin accompanied by a prominent eosinophilia and elevated serum IgE levels are common features of human infection with filarial parasites. Recent finding have shown the role of IL-4, IL-5 and IFN-γ in the induction of hypersensitivity response (140).

Eosinophilia, mastocytosis and increased IgE synthesis, all appear to be induced by cytokines from the TH-2 subset of CD4+ T cells; IgE production is

stimulated by interleukin-4 (IL-4), eosinophils by IL-5 and mastocytosis by IL-3 and IL-4 (141). Recently, Allen et al have reported some interesting finding in *Brugia malayi* that specific T-cell hyporesponsiveness and depressed antibody production is a key feature of human infection with the filarial nematodes. *Brugia malayi* and *Wuchereria bancrofti.* Despite this immune suppression, responses indicative of Th-2 subset activation are present, including unusually high levels of specific IgG-4. It was tested to see the possibility that infection with filarial nematodes causes a reduction in the co-stimulatory or antigen-presenting capacity of macrophages resulting in a failure to activate specific T-cells. Adherent peritoneal exudate cells (PEC) from mice implanted with adult *B. malayi* were used to present antigen to the specific T-cell clone, D10.G4. Proliferation of the D10 cells at even background levels was completely blocked by the presence of implant derived adherent PEC. However, cytokine production by these cells in response to antigen was intact, and thus PEC from implanted mice are capable of functionally processing and presenting antigen. The elicitation of a suppressive cell population was specific for live adults as cells from mice implanted with dead adult parasites effectively stimulated D10 proliferation. The block in cellular proliferation is not due to the production of factors typically associated with macrophage suppression such as nitric oxide or prostaglandin's. These observations are consistent with the T-cell hyporesponsiveness seen in human cases of patent Brugia infection and may provide a murine model for the immune suppression seen in lymphatic filariasis (142)

2.3.2 Humoral Responses.

It is known that a multitude of antigens are shared not only by different stages of a given filarial parasite but also by filarial worms belonging to different

species. More recently, it has become evident that distinct developmental stage of filarial worms also possesses unique, stage-specific antigens in addition to "public" antigens shared by many stages of parasite (143). The development of antibody responses to soluble antigens of *Brugia malayi* microfilariae was studied in a cohort of adults who moved into an endemic area without previously been exposed to filarial helminths (144). Filariasis can be classified into three groups viz. endemic normals (EN), microfilaremics (mf+ve) and elephantioids (chronic patients) (CH). The immune status of these three groups was examined in terms of:

(i) Specific antibody levels;

(ii) Ability to induce antibody dependent cellular cytotoxicity (ADCC) to microfilaria antigens by immunoblotting.

As measured by ELISA with *Brugia malayi* microfilaria antigen, many endemic normal sera and most elephantiasis sera exhibited strong cytotoxicity against *W bancrofti* microfilariae, whereas none of the mf+ve sera had any such activity. Immunoblotting studies revealed that a 79-kDa protein was consistently recognised by sera from all endemic residents. Endemic normal sera and elephantiasis antoid sera, which exhibited maximum cytotoxicity, together specifically recognised three proteins with molecular weights 25, 58 and 68 kDa and these three proteins could belong to the group of candidate antigens that induce resistance to filarial infection (145). Hussain et al have classified the IgG antibody in human filariasis into four subclasses. IgG have been distinctly classified according to their structure, function and degree of participation in antibody response to complex antigens. They have analysed both quantitatively and qualitatively the filaria-specific IgG sub class response of 20 patients with lymphatic filariasis presenting either with chronic lymphatic obstructive pathology and elephantiasis (CP) or with asymptomatic microfilaremia (MF). Sub class specific monoclonal antibody was used in an enzyme linked immuno-

sorbent assay to detect IgG filarial antibodies quantitatively and in immunoblot analyses to determine qualitatively the subclass antibody specificities. Qualitatively, the most significant differences among the patient groups were in the levels of IgG4 which were more than 17 times higher in MF patients (geometric mean 64.7μg/ml) than in those with CP (mean 3.7μg/ml). When qualitative analyses were done on the same sera major differences were noted particularly in the recognition profiles of the IgG1, IgG3 and igG4 responses. IgG1 and IgG4 class of antibodies to antigens with molecular weight grater than 68 kDa in all patients with elephantiasis, where as MF patients showed most of their reactivity to antigens small than 68 kDa. For IgG4 and MF patients showed prominent recognition of antigens throughout the entire range of m.w. where as those with cp had very little IgG4 recognition of antigens, Of any m. w. interest, this relationship was essentially reversed in the IgG3 antibody responses (especially to antigens > 68 kDa) and to a lesser extent the IgG1 responses. These findings demonstrate correlation's of potential cause/effect significance between IgG4 antibody responsiveness and the Immunomodulated asymptomatic MF form of clinical filariasis and between IgG3/IgG1 antibody responsiveness and the clinical presentation of Cp (146).

In a study by Ottesen et al (147) sixty eight individuals from a Pacific island hyperendemic for sub periodic bancroftian filariasis were selected from a larger study population to include the entire clinical spectrum of filarial infection in that region and also "endemic control" group without clinical or parasitologic evidence of filarial infection. Analysis of the leukocytes and humoral immune responses yielded the following major findings:

1. Ig levels of specific antifilarial antibodies of three different immunoglobulin classes (IgG and IgM measured by ELISA and IgE determined by radioimmunoassay) were significantly greater in the "endemic control"

population than in the patients with filariasis, an observation which is true for both children and adults.

2. The endemic controls also had significantly higher levels of serum IgM and C- than the filariasis patients.

3. While individuals with "filarial fevers" and "chronic (lymphatic) pathology" did have significantly lower IgG antibody responses to filarial antigen than the controls, the lowest antibody levels were found in the patients with microfilaremia.

4. Symptomatic patients (i.e. those with filarial fevers or lymphatic obstruction) regularly showed higher specific antibody responses to filarial antigens than symptomatic, infected individuals, although the difference did not reach statistical significance. These finding are in concert with previously reported, intriguing observation that lymphocyte proliferative responsiveness to filarial antigens was much greater in individuals of the "Non-infected" endemic control population than in patients with filariasis; furthermore, they indicate the important issues that must be approached and resolved to define the immunologic determinants leading both to the various filarial clinical syndromes and to protective immunity (147).

In an interesting study Kwan-Lim et al have compared filarial specific humoral immune response of adult residents of two areas of Papua New Guinea who differed in transmission of *Wuchereria bancrofti* infection. The majority of residents of the village of Bonahai, in an area where transmission of filariasis had been interrupted for 20 years by insecticide spray program intended to control malaria, showed no parasitologic signs of *W. bancrofti* infection and were negative for both circulating phosphoryl choline antigen and peripheral blood microfilariae. In contrast, adult residents of the village of Nanaha were in an area exposed to infection, and were phosphorylcholine antigen and microfilariae

positive. The antibody response of these two groups to both adult worm excretory/secretory (ES) antigen and somatic antigen extract was examined to determine which components of the filarial specific immune response were dependent on active infection. Identification of these immune responses may point to immunologic methods to evaluate control program for lymphatic filariasis. Adult from Bonahai were found to have significant immune responses to methionine labelled ES Ag by immunoprecipitation and to adult somatic antigen extracts by ELISA and immunoblotting. This result is consistent with the fact that these individuals were previously exposed to and/or infected with *W.bancrofti parasite*. Similarly, residents of the endemic village had detectable immune responses to these Ag irrespective of their microfilaremic status. The most striking immunologic difference observed between the two groups was that the residents of Bonahoi had a dramatically reduced filarial specific IgG4 antibody response to both adult somatic Ag and adult ES Ag. These data suggest that longitudinal measurement of filarial specific IgG4 levels may be a useful seroepidemiologic indicator of changes in *W.bancrofti* infection status (148).

The four sub classes of IgG are distinct in structure, function and degree of participation in the response to complex antigens. Because these differences could have auto pathogenic significance, they analysed total antigen specific IgG of each subclass in 31 patients with different clinical manifestation of Bancroftian filariasis. Subclass specific, affinity purified polyclonal antibodies were prepared from antisera raised in sheep immunised with purified myeloma IgG subclass proteins. These were radiolabeled (I-125) and IgG4 in solid phase radioimmunoassays (SPRIA). The antigen-specific SPRIA was used with *Brugia malayi* adult antigen measurement (BmA) bound to sepharose 4B, whereas measurement of total IgG subclass level in each serum was with goat antihuman

IgG bound to the solid matrix. Quantification of total subclass levels was by referencing to the WHO 67/97 standard, and of specific subclass antibody by development of standards from high tittered sera.

Although there was modest increase of total IgG1 and IgG2 in patients with filariasis compared with normal the most striking findings was the extreme elevation of both total IgG antibody response. IgG4 antibody titre was particularly higher (up to 90%) in patients with either microfilaremia or with tropical pulmonary eosinophilia (TPE) syndrome. The meaning of this special prominence of the IgG4 antibody response to filarial infection is not yet clear but the question whether these antibodies play a role in immediate hypersensitivity reactions or blocking antibodies is being investigated for its potential pathogenic significance (149).

Lobos et al (150) states major allergen of the human filarial parasite *Brugia malayi* has been identified by two dimensional immunoblot analysis using a serum pool from patients with tropical pulmonary eosinophilia. The allergen is composed of two Ag with molecular weight 25 and acidic iso-electric point (BM 23-25). Immunoblots using affinity-purified IgE antibodies to BM 23-25 indicated that BM 23-25 is expressed mainly in the microfilarial stage. Digestion of the allergen with endo-glycosidases indicates that it has N-linked oilgosaccharide chains. Analysis of the reactivity of 'T' cells derived from patients with lymphatic filariasis revealed that the BM 23-25 allergens was capable of stimulating 'T'cell proliferation BM 23-25 was also shown to induce IgE production in-vitro from PBMC derived from patients with either TPE or other filarial symptoms. Broncho-alveolar fluid of patients with TPE contained IgE antibodies the recognised BM 23-25 strongly. An observation suggesting that the macrofilarial allergens might be involved in the pathogenesis of the TPE syndrome (150).

Antibody levels to Ag extracted from adult worms were determined for each of the IgG subclass IgM and IgE. The dominant isotypes of antifilarial antibodies was IgG4, which represented 88% of the total IgG in asymptomatic microfilaremic, most of whom possessed 100 to 1000 μg/ml of specific antibody of this subclass, geometric mean 762 μg/ml. Patients with chronic disease (elephantiasis), who were generally amicrofilaremic, had substantially high levels of IgG1, IgG2 and IgG3 but a 3.4 fold lower geometric mean level of specific IgG4 (222 μg/ml) than asymptomatics with or without microfilaremia. In contrast, specific IgE antibody levels in cases of elephantiasis were on average 4.5 times higher than those found in the asymptomatic carrier state. The majority of microfilaremics were therefore typified by extremely high specific IgG4 concentrations and relatively low IgE reactivates, whereas of clinical cases tended to show the reverse relationship (151). Yazdanbaksh et al (152) says lymphatic filarial infection in humans is associated with strong skewing of the immune response towards the Th2 arm, with prominent interleukin-4 producing cells and elevated level of immunoglobulin G4 (IgG4) and IgE antibodies in peripheral blood. To determine how such generalised TH2 imbalances govern responses to individual parasite antigen, the profiles of Isotypes of antibodies to two recombinant proteins of *Brugia spp* were studied one molecule was C-terminal portion of proteins of the filarial heat shock protein 70 (BpG-26) representative of a cyptoplasmic protein and the second antigen was a single unit of the tandem repeats of a *Brugia* polypeptide (BPL-4), a secreted product which is prominently exposed to the immune system. In the serum samples the level of IgG1 and IgG3 responses to both BPa-26 and BPL-4 were high, IgG4 and IgE antibodies to only BPL-4, not to be BPa-26, were prominent. Thus an antigen which is chronically exposed to the immune system elicited a TH-2 dependent isotype switch, as manifested by increased IgG4 and IgE responses. Moreover

IgG4 and IgE responses to BPL-4 showed a strong negative association, suggesting that mediators other than interleukin-4 must be responsible for such differential regulation of these two isotypes. When the data was analysed as a function of clinical status, a striking association between elevated levels of IgG3 antibodies to BPa-26 and manifestation of chronic obstructive disease was found; elephantiasis patients showed significantly higher levels of IgG3 antibodies to BPa-26 than microfilaremic and asymptomatic microfilaremic.

This indicates that an imbalance of isotypes of antibodies to particular filarial antigens might play a role in the pathogenesis of chronic disease (152). In other interesting paper Garraud et al (153) enumerated that filarial infection is characterised by an immune response associated with the class of antibodies and secretion of production of Ag specific IgG4 and IgE and secretion of IL4 and IL5. To identify filarial Ags capable of inducing such responses and to analyse the role of Ags themselves to play in sustaining it, 24 recombinant filarial parasite proteins were screened for their ability to be recognised by sera from 67 individuals with tissue-invasive filarial infection. Among the recombinant proteins that were recognised by IgG4 IgE Abs in 25% of the sera or more, two were selected on the basis of their ability to elicit polyclonal and Ag specific IgE/IgG4 antibodies in vitro ov27 (analogous to ov7/cystain, a cysteine protease inhibitor) and ovD 5B(analogous to ov33, an aspartyl protease inhibitor) induced both a polyclonal and Ag specific IgE/IgG4 responses that was blocked by neutralising Abs to IL-4 and IL-13 or by soluble IL-4 receptors. Recombinant human IFN-γ and IL-2 also led to decrease in the production of polyclonal and Ag specific IgE/IgG4 Abs. In addition, these two recombinant proteins preferentially stimulated the secretion of IL-4, IL-5 and IL-10 (in contrast to IFN-γ). The data suggested that certain epitopes on filarial Ags preferentially elicit a

Th-2 type response and provide an in *vitro* model for dissecting the mechanisms underlying this preferential response (153). King (154) reported that parasite Ag added to cultured lymphocyte cultures significant expansion in the number of Ig- secreting B cells secreting Bc for IgE and all IgG subclasses. In contrast, addition of a non-parasite Ag like tetanus toxoid generated increased Fo of Ig - secreting Bc for IgG1 and IgG2 or IgE 3 and on expansion of IgG4. The essential role of IL-4 in expansion of IgE and Ig4 secreting B-cells in response to filarial antigen was demonstrated when simultaneous addition of neutralising anti IL-4 antibodies completely inhibited this response was studied. Neutralising anti IL-4 had no effect on either filarial or tetanus toxoid driven expansion of IgG1, IgG2, IgG3-secreting Bc. An inhibitory role of endogenously produced IFN-γ was also shown when addition of neutralising anti-IFN-γ to cultures significantly augmented Ag driven expansion of Bc-secreting IgE and all IgG subclasses; IgE, 0.4 to 9 fold, 1.4 to 12 fold; IgG1, 1.6 to 13 fold; IgG2, 1.9 to 5 fold; IgG3, 2.7 to 6 fold. This study demonstrated that parasite Ag stimulates Bc expansion in helminth infected patients. Although this response for all five isotypes studied is down regulated by IFN-γ the generation of only the IgE and IgG4 responses appear to be mediated selectively by IL-4. These findings support the concept that IgE and IgG4 production are linked and related to the quantities of IL-4 and IFN-γ induced by Ag specific 'T' cells (154).

King et al (155) has tried to spot out whether there is a reciprocal relationship between IL-4 and IFN-γ production in persons with parasite induced elevations in serum IgE. PBMC were obtained from helminth- infected individuals with a broad range of serum. IgE levels that fell into two distinct groups: extremely elevated (HI; range, 4,129-18,400 ng/ml) or elevated (EL; range 403-1,018 ng/ml), relative to control subjects (NL; range, 4-159 ng/ml).

PBMC were stimulated *in vitro*, with Ag or mitogens and IL-4 and IFN-γ were measured by ELISA in culture supernatants. Helminth Ag (but not tetanus toxoid) stimulated IL-4 production in eight of nine HI (range, 49-150 pg/ml) but was undetectable in either EL or NL ($p<0.01$). In contrast, PBMC from EL produced level of IFN-γ to helminth Ag (Gm=358 pg/ml) compared with HI (Gm=89pg/ml; P=0.02) and NL (Gm=9 pg/ml; $p<0.001$). Tetanus toxoid induced comparable levels of IFN-γ among the three groups. Mitogen driven IL-4 production was nine fold greater in HI geometric mean (Gm=953 pg/ml) versus EL (Gm=111 pg/ml; $p<0.01$) and NL (Gm=193 pg/ml; $p<0.001$) and correlated with serum IgE levels ($r=0.8$; $p<0.01$). Mitogen driven IL-4 production was nine fold greater in HI geometric mean (Gm)=913 pg/ml versus EL (Gm=111 pg/ml; $p<0.01$) and NL (Gm=193 pg/ml; $p<0.001$) and correlated with serum IgE levels ($r=0.8$; $p<0.01$). Mitogen driven IFN-γ synthesis was equivalent among the groups. Although parasite Ag driven IL-4 secreting $CD4^+$ cells were detected (by ELISA) among infected subjects with both high and low serum IgE levels, the number of IL-4 exceeded that of IFN-γ secreting cells among individuals with elevated serum IgE levels, whereas the opposite relationship existed among subjects with normal serum IgE. In a sub population of infected individuals (n=4), parasite-Ag added to PBMC cultures induced polyclonal IgE that was directly associated with the parasite Ag driven IL-4 production and inversely related to IFN-γ synthesis in PBMC supernatants from parallel cultures further more neutralising anti IFN-γ antibody augmented both parasite driven IL-4 synthesis and IgE production *in vitro* (n=4). The data indicate that helminth induced serum IgE levels are directly related to an increased capacity by PBMC to produce IL-4 and inversely associated to IFN-γ production. It further supports the concept that IL-4 and IFN-γ reciprocally regulate IgE *in vivo* (156).

Mohanty et al (157) has investigated the relationship of antifilarial IgG4 and IgE to the intensity of transmission and duration of filarial infection in an endemic population. Antifilarial antibody levels in children residing in a village in Papua New Guinea, where transmission of *Wuchereria bancrofti* was reduced by repeated insecticide spraying, were compared with that of residents of three nearby village, where no control measures had been used. Antifilarial IgG4 levels were significantly lower in children from the former village in comparison to the later ($P<0.01$) and correlated with age ($p<0.05$) and intensity of microfilaremia ($p<0.01$). In contrast antifilarial IgE was elevated to similar levels in children and adults from both the villages. Antifilarial IgG4 (and not IgE) levels in endemic population appear to be directly related to the duration of infection or to the cumulative exposure to infective vectors (157).