background to the work

Infection with *Wuchereria bancrofti* parasite provokes a range of immunological responses in human host, the outcome of which determines the clinical status of infection [156]. In an endemic area for bancroftian filariasis a large number of people who despite life long exposure to infective larvae through repeated mosquito bite do not show detectable microfilariae in their blood and have no apparent symptoms of filariasis. They are classified as endemic normals (EN) and are believed to be putatively immune [158, 159]. In terms of filaria antigen specific B-cell and T-cell responses, these endemic normals exhibit more intense immune response than that of microfilaremic individuals, which is often comparable to that of individuals with chronic lymphatic dysfunction [156, 160-164]. Then one can ask in what way the immune response of endemic normals is different from that of other clinical groups in bancroftian filariasis and whether all the endemic normals are truly immune and protected from infection.

The principal difficulty in characterising the protective immune responses in endemic normals is our inability to ascertain who is truly infection free and therefore putatively protected. A large number of endemic normals may actually have cryptic infection or they might have cleared the infection recently and therefore appear normal. Such individuals can not be categorised as truly endemic normals or individuals free of infection. Some individuals who have cryptic infection may be having very low levels of circulating microfilariae, which can not be detected by the conventional detection system. However, the adult worm, which lives in the tissues, would secrete some antigens into the blood circulation. Therefore, the detection of these antigens in blood may reveal the active infection status of the individuals. Based on these assumption two monoclonal antibody based enzyme immunoassays for antigen detection (AD12 and Og4C3 ELISA) specific for *W.bancrofti* microfilarial and adult worm antigen

has been developed [165, 166].By using these antigen detection systems, it has become possible to identify two populations of asymptomatic amicrofilaremic individuals: those who have no clinical symptoms and no parasite circulating in the blood but are positive for circulating antigen and presumably carry adult worms, and the others those who besides having no clinical symptoms and no parasite are also negative for circulating antigen and presumed to be infection free. In the present study, we have used one such antigen detection system to monitor the infection status of endemic normal individuals.

All the three categories of individuals in bancroftian filariasis have high levels of parasite antigen specific IgG4 antibodies circulating in their blood with the asymptomatic microfilaremic individuals having very high levels in comparison to the endemic normals and patients with chronic lymphatic obstruction [167]. The antigen specific IgE titres are also elevated in these clinical groups but in an inverse manner to the IgG4 titres [167]. It is known that the cytokine milieu in which the immune system encounters an antigen determines the nature of the immune response mounted against it and the relative proportion of IFN-γ and IL-4 present during the early stages of antigen presentation polarises the immune response either to Th1 type or Th2 type [168, 169]. Therefore, the differential immune response observed in endemic normals, asymptomatic microfilariae carriers and chronic filaria patients must be arising due to different cytokine profiles generated in them by the parasite antigens. In an attempt to understand the mechanism underlying this phenomenon in an interesting study by O. Garraud, et al screened 24 recombinant filaria antigens from *Onchocerca volvus, Loa loa,* and *Brugia malayi* [170]. It was observed that two antigens namely Ov27 and OvD5B induced IgE and IgG4 responses and preferential secretion of IL-4, IL-5 and IL-10 in contrast to IFN-γ secretion *in vitro,* in the peripheral blood lymphocytes of *Loa loa* and *Onchocerca volvus* infected

individuals. This study suggested that certain epitopes present on filarial antigens may preferentially elicit a Th2 type immune response in humans and therefore such antigens can be used as *in vitro* model for dissecting the mechanism of differential immune response in filariasis. But no data were available on how the lymphocytes from endemic normals behave in the presence of these antigens. Another study using recombinant antigens of *Brugia* spp. identified one antigen namely BpL-4 which was recognised by the IgE and IgG4 antibodies present in the sera of filaria patients [171]. It appeared that antigens which are chronically exposed to the immune system elicit Th2 cytokine dependent isotype switch in filaria patients. There has been other studies which have attempted biochemical characterisation of an IgE inducing antigen of *Brugia malayi* adults by two dimensional immunoblot using pooled sera from tropical pulmonary eosinophilia (TPE) patients [172] or identification of *Brugia pahangi* adult antigen fraction which can induce differential proliferation and IL-10 secretion in the lymphocytes of individuals with chronic lymphatic dysfunction [160]. The role of suppressive cytokines, such as IL-10 and TGF-β which may be controlling parasite antigen specific anergy in asymptomatic microfilaremic individuals have been studied [173].

All of them have yielded significant and valuable information regarding how the imbalance of different antibody isotypes to specific filarial antigens and certain cytokines might be regulating the pathogenesis of filarial disease. From all the studies carried out so far it is clear that a systematic search using suitable filaria antigens and lymphocytes from endemic normals, asymptomatic microfilariae carriers and filaria patients would throw significant light on how the observed differential immune response is generated.

Out of the three categories of individuals found in an endemic area the

endemic normals and asymptomatic microfilaria carriers are the most interesting; both the groups are asymptomatic, but immunologically they behave differently. IgG4 levels are very high in asymptomatic microfilaremic individuals whereas the same is much lower in endemic normals suggesting that live microfilaria induces higher levels of parasite antigen specific IgG4 antibody synthesis [174]. Production of IL-2 and IFN-γ in response to parasite antigens in microfilaremic individuals is impaired while these two cytokines appear to be unregulated in disease free individuals [175]. Therefore, analysing immune response of endemic normals that are free of infection as well as individuals with cryptic infection, who appear to be normal, and comparing them with that of asymptomatic microfilaremic individuals may give us some idea about the nature of protective immunity in filariasis.

In the case of chronic infections like lymphatic filariasis it is difficult to identify whether one individual is infection free or not without reasonable longitudinal observation and proper biochemical, parasitological and clinical examination.

The individuals once declared amicrofilaremic do not necessarily remain infection free, they may show microfilaria some other time or may develop filarial pathology like chronic/acute patients who are also generally amicrofilaremic. In order to establish the fact that the endemic normals are infection free, it would be ideal to follow-up their clinical and parasitological status at regular intervals for a reasonably long period. Often it is difficult to detect circulating microfilaria (MF) if their levels are very low and also if mf are cleared recently by the immune system. In that case it is advisable to look for circulating parasite antigens in the blood. The live lymph dwelling adult worms would be secreting antigens into the blood circulation. Till now there are two antigen detection kits namely Og4C3 and AD12 ELISA available [165, 166].

Though the monoclonal antibodies used in these two kits are directed against heterologous parasites they cross-react with specific antigens of *W.bancrofti* parasite. Sometimes individuals having active parasite infections may not have those specific antigens circulating in their blood. It is quite possible that some other antigens would be circulating. Since presence of live parasite correlates with IgG4 level we measured and monitored the levels of IgG4 antibodies directed against mf total antigens. Thus before including any individual as endemic normal in this study their clinical and parasitological status as well as mf specific IgG4 levels were monitored continuously at 3 months intervals for a period of 3 years. Individuals who did not have circulating microfilariae and had no clinical symptoms of filariasis, their sera were screened for circulating *W.bancrofti* microfilarial and adult antigens. Those who showed no clinical symptoms, had no circulating microfilariae as well as circulating *W.bancrofti* antigens, in spite of living in the endemic area for a long time, since their birth, and had low levels of IgG4 (<10,000 titers) were classified as truly endemic normals. Those who were having circulating microfilariae as well as antigen in their blood but had no clinical symptoms of filariasis and high levels of IgG4 (>30,000 titres) were observed for three months with their consent to ensure that they continue to harbour microfilariae without showing any clinical symptom and continue to have high levels of IgG4 before inclusion as asymptomatic microfilaremic individuals (ASM) in this study. After taking blood from these ASM individuals they were given anti-filarial treatment by a qualified physician.

In the present study the antigens used include excretory-secretory molecules of *W.bancrofti* microfilariae (mf-ES) obtained by culturing live mf for 24 hours, soluble antigens of *Setaria digitata* (a cattle parasite showing strong antigenic reactivities with bancroftian filaria sera) adults [176], and purified protein derivative (PPD) from *Mycobacterium tuberculosis* as a control antigen. As

W.bancrofti mf were scarce, *S.digitata* adult soluble antigens were used for detail analysis in the present study. These antigens were used for *in vitro* stimulation of lymphocytes, collected from the endemic normals, asymptomatic microfilaremic carriers, and chronic filaria patients, for secretion of cytokines e.g., IL-2, IL-4, IL-5, IL-10, and IFN-γ.

In a study of leprosy patients Barry Bloom has suggested that protective response can be defined by measuring the cytokine profiles in different clinical groups [177]. In leprosy the lymphocytes isolated from tissue specimens were used for studying cytokine profiles but in the present study we have used peripheral blood lymphocytes from individuals infected with filaria parasite to study the same. Measuring the levels of the cytokines in the serum will not be very meaningful since it will give the levels of cytokines secreted by immune cells over a long period of time, therefore stimulating PBMC *in vitro* in the presence of filaria antigens for secretion of cytokine was only option left to us. In this study we have measured cytokine profiles in the lymphocytes of individuals belonging to different clinical groups in response to SDS-PAGE fractionated *S.digitata* adult soluble antigens as well as *W.bancrofti* mf-ES antigens. In a study by Dimock, et al. using SDS-PAGE fractionated antigens of *Brugia pahangi* adults it was found that antigens of different molecular weights induced differential proliferation and secretion of IL-10 in the lymphocytes of individuals with chronic lymphatic dysfunction [160]. Similarly use of fractionated antigens of *Mycobacterium leprae* to stimulate peripheral blood lymphocytes of leprosy patients has been reported [178]. However, no differential Th1/Th2 response was observed. But in another study when Dockrell, et al. tested the ability of 20 mycobacterial antigens to induce differential IFN-γ secretion, they were found to do so in the lymphocytes of tuberculoid leprosy patients [179]. All these studies prompted us to use fractionated antigens to study the cytokine profiles in the individuals belonging to different clinical groups.