Background to the work
Lymphatic filariasis is a chronic human disease affecting about 120 million people living in 73 tropical and subtropical countries of the world. Conservative estimate indicates that about 1.2 billion people living in those countries are at risk of getting infected. Since, the disease is not a fatal one, it has not attracted much attention worldwide. But the disease is an immunologist's enigma. The parasites are one of the most complex organisms, which causes disease in the humans. It has three distinct life stages and the infective form is transmitted by mosquito. For its survival the parasite requires another organism viz. \textit{Wolbachia}, which it carries within its body. It appears that if one can get rid of \textit{Wolbachia} from the parasite by treatment with antibiotics like tetracycline or chloramphenicol, then the parasite can not survive. Effective chemotherapy is available but diagnosis of infected persons who are living in an endemic area is the limiting factor in initiating treatment. A large proportion of individuals living in an endemic area, who inspite of getting exposed to the parasite through repeated mosquito bite, are free of infection. We do not have a clear-cut picture about the nature of protective immune response, if any mounted by them or the nature of the parasite antigens capable of inducing such responses. There is no animal model available which would make the study of host-parasite interaction more amenable to analysis. Though valuable informations have been obtained by infecting animals, which are not permissive to infection, these informations can not be directly extrapolated to humans.
The work embodied in this thesis focuses on understanding the immune response of truly infection free individuals living in an endemic area to live *Brugia malayi* adult and infective larvae. We have compared the immune response of truly infection free endemic normals with that of asymptomatic microfilaremic individuals living in the same endemic area and also with non-endemic normal individuals living in areas where transmission of the disease does not occur. We have taken utmost care to select our study subjects on the basis of stringent epidemiological, clinical, parasitological and immunological parameters, so that only the true endemic normal individuals who have been exposed to the parasite but subsequently cleared the infection are included. For identification of such truly infection free individuals we have followed them up longitudinally monitoring their clinical, parasitological and immunological status for 3 years. The asymptomatic microfilaremic (ASM) individuals were also identified from residents of the same endemic area on the basis of presence of circulating microfilariae in their blood and other immunological parameters. The non-endemic normal individuals were selected from residents of a population living in an area in Leh, which is more than 10,000ft above sea level. Thus in the present study attempts have been made to identify the nature of immune response which might be keeping the endemic normal individuals free of infection.

In any study of this nature involving human subjects the major problem is the formation of different groups, which are clearly defined on the basis of their clinical, parasitological, and
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immunological status. This has to be done taking into consideration the genetic make up of the individuals comprising the groups as well as their nutritional status, and various other factors, which may contribute to the diseased state.

Filarial infection is initiated when a female mosquito after taking a blood meal deposits infective larvae (L3) on the skin of the individual she is feeding on. The L3 then enters the tissue of the host through the hole, drilled by the mosquito for feeding, and finds its way to the nearest lymph node where it develops into an adult. If fertile adults of both the sexes are there then they mate and produce microfilariae, which circulate in the peripheral blood mostly during night. Since L3 is the first of the three different developmental stages of the parasite, which an uninfected host encounters the interaction of the host immune system with the live L3 is the singular most important event, deciding about the establishment of infection. The cytokine milieu that the incoming L3 encounters may be responsible to a large extent for the subsequent fate of the L3. Another important stage in the life cycle of the parasite is the adult, which can stay within the lymphatics for almost a decade producing microfilariae continuously. In order to survive, the adult must be able to suppress the host immune response towards itself. Therefore, we have focussed on the L3 and adult stages of the parasite. Since we wanted to study the interaction between the parasite stages and the host's immune system as close to the natural condition as possible, we have used live parasites in our study and co-cultured them in the presence of
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PBMCs obtained from endemic normal, asymptomatic microfilaremic and non-endemic normal individuals.

From our study it appears that as L3 enters inside an unexposed individual it induces secretion of IL-10, IL-12, IFN-γ and IL-1β, which is more like an inflammatory response, which may be beneficial for the parasite at the initial stages of establishment of the infection. At some later stage the response is skewed towards a type, which involves predominant secretion of IL-4, which may be required for survival of the adult inside the host. Those individuals who continued to secrete IFN-γ predominantly in response to the parasites may be the individuals, who later clear the infection, and become the so-called EN. This model requires further elucidation by looking into the cellular types targeted by the parasites and designing more studies involving human subjects and live parasites. Our study suggests that the soluble antigen and the live parasites are perceived by the immune system of the humans quite differently.

We have not only looked at the kinetics of cytokine secretion by the PBMCs of the study subjects in response to live parasite or their soluble filarial antigens but also looked into the number of cells producing a particular cytokine by means of internal cytokine staining and visualizing them by FACS. The expressions of surface receptors of those cytokines have also been measured. The cytokine secretion pattern of IL-4, IL-10 and IFN-γ correlated very well with their internal profile as well as their receptor expression on the surface of the lymphocytes by all the three groups of
individuals studied. Our study also showed that lymphocytes of infection free EN individuals when cultured with live L3 responded very strongly in comparison to when they were cultured with live adult or BmA as seen by the expression of lymphocyte activation markers such as IL-2 receptor-\(\alpha\), CD69, CD45RO and HLA-DR molecules. The lymphocytes of NEN and ASM individuals did not respond that strongly to live L3 or any other antigen.